# ABSTRACT


Title of Thesis: PLANT BIOMASS ALLOCATION AND COMPETITIVE INTERACTIONS IN COASTAL WETLANDS OF THE CHESAPEAKE BAY: EXPERIMENTAL AND OBSERVATIONAL STUDIES

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Wetlands are diverse environments whose vegetation provides numerous natural services including flood and erosion control and excess nutrient absorption; however conditions created by sea level rise and nutrient pollution could lead to possible wetland loss. To assess the responses of vegetation in these conditions in the Chesapeake Bay tidal wetlands, an observational study and a competition greenhouse study were conducted. An aboveground to belowground biomass relationship assessment was done along a salinity gradient within a subestuary of the Chesapeake Bay. The competition study focused on the relationship between a native and a non-native species in the aforementioned conditions. These studies revealed *Phragmites australis* had more biomass and lower rooting depths than the native *Spartina cynosuroides* under varying conditions of salinity, competition, and fertilization. Growth of *S. cynosuroides* may facilitate the growth of *P. australis* through salinity uptake and root aeration, which could have important impacts on wetland management practices.

# PLANT BIOMASS ALLOCATION AND COMPETITIVE INTERACTIONS IN COASTAL WETLANDS OF THE CHESAPEAKE BAY: EXPERIMENTAL AND OBSERVATIONAL STUDIES

by

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# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# CHAPTER 1

## *Introduction*

Wetlands are ecologically productive and diverse environments that have important ecosystem function and value. The vegetation within these environments assists in flooding and erosion control in coastal environments, as well as act as a natural filter for excess nutrients (Mitsch and Gosselink 1993). This is an important characteristic of wetlands, because high nutrient run-off can cause eutrophication in water systems, which can be detrimental to the ecosystem. The vegetation within the wetland system adds organic matter to the soil, which allows for wetlands to counteract natural erosion and compaction.

Plant stress in wetlands is caused by a variety of factors including, but not limited to salinity and inundation. Depending on what stressors are present growth may need to be compensated with different mechanisms to allow the plant to survive. For example, the conditions predicted from accelerated sea-level rise may lead to increased salinity and inundation levels which could be major stress factors (Spalding and Hester 2007). Increased salinity levels can be toxic to fresh water plant species and some oligohaline species. As summarized in Howard and Mendelssohn (1999a), salinity causes stress in vegetation by the accumulation of ions and toxins in the soil, high water stress from increased osmotic potential in the root zones, and the buildup of ions within the plant tissue. Additionally, the increase in flooding frequency will decrease soil oxygen concentration and as a result create an anoxic soil environment. Anoxic soil conditions are stressful growing environments for many plant species (Pezeshki 2001) because nutrient uptake in root systems is dependent on oxygen concentration in the soil (Morris

and Dacey 1984) and root respiration. Each of these stressors alone can decrease the biomass production in the plant species. Together these two stressors can limit plant production due to the energy used to diminish the effects of the unfavorable conditions (McKee and Mendelssohn 1989, Howard and Mendelssohn 1999a, Spalding and Hester 2007). By not producing high amounts of biomass, there will be less organic material inputs, which could result in slower accretion rates within these environments. Lower biomass production could also result in lower soil retention capabilities and erosion control. Higher erosion rates, like those predicted in Jamaica Bay, NY (Hartig et al. 2002), could lead to lower elevations in wetlands and eventual subsidence.

Wetland environments are able to adapt to natural sea-level rise with natural accretion (Morris et al. 2002). However with the predicted accelerated rates of sea-level rise in the Mid-Atlantic region (Titus et al. 2009), the natural wetlands could be lost due to low accretion rates and decreases in elevation (Poret-Peterson et al. 2007) or higher erosion rates (Hartig et al. 2002, Titus et al. 2009, Mariotti et al. 2010).

Nutrients are often seen as beneficial to plant growth and can help ameliorate stressors like salinity and inundation (Linthurst and Seneca 1981, Howes et al. 1986). Excess nutrients, like nitrogen and phosphorus, are added to the coastal wetland environments from a variety of anthropogenic sources including agricultural inputs and urban outflows. The increase in fertilization may cause plants to shift from production of belowground biomass to aboveground biomass, because of the decreased need for belowground systems to forage for nutrients, thus decreasing inputs of organic matter and lowering retention capabilities (Valiela et al. 1976, Minchinton and Bertness 2003, Turner et al. 2004). By reducing belowground biomass, the structure of the wetland soils

may be compromised, so if the vegetation dies the wetland could turn to open water (Mendelssohn et al. 1981). This could cause similar accretion and erosion problems as the stress caused by increased salinity and flooding (Mendelssohn et al. 1981, Delaune et al. 1994).

In addition to sea-level and nutrient stressors, diverse coastal wetland communities are threatened by the invasion of highly competitive non-native species. Studies have shown that invasions of species, such as *Phragmites australis* (Cav.) Trin. ex Steud., invade in disturbed wetlands; for example, wetlands that have been subject to increased salinity, flooding, and excess nutrient inputs (Rice et al. 2000, Burdick and Konisky 2003, Alvarez-Rogel et al. 2007, Chambers et al. 2008). Specifically *P. australis* is a high-biomass-producing species that creates widespread rooting systems and dense monoculture stands once it invades (Chambers et al. 1999, Rice et al. 2000, Saltonstall and Stevenson 2007). Once *P. australis* is established it often outcompetes and shades out native species destroying the native ecosystem diversity (Haslam 1971). Many state governments within the United States deem *P. australis* as a pest and work hard to eradicate it (Rice et al. 2000) including the Chesapeake Bay region.

In order to understand the dynamics of vegetation responses in predicted coastal wetland environments, an observational study and a competition greenhouse experimental study were conducted. To assess the biomass production in example coastal wetlands of the Chesapeake Bay, a biomass collection study was conducted on a salinity gradient along the Nanticoke River, a subestuary of the Chesapeake Bay. This study is fully described in chapter two. Biomass samples were taken to determine the relationship between the aboveground and belowground biomass production and the

effects of salinity on production. Additionally, species information was taken at each site to evaluate if salinity affected the abundance and diversity of species. To assess the responses of vegetation and competition to predicted conditions of sea-level rise and increased nutrient pollution, an experimental competition study was performed between a native and non-native species. The experiment focused on the competition between the species by assessing the biomass production of each species. The treatments were designed to mimic predicted sea-level rise and nitrogen polluted conditions. Chapter three will explain in detail this greenhouse competition experiment. These studies, summarized in chapter four, aimed to provide information on the responses of wetland environments to the predicted conditions of sea-level rise and nitrogen pollution in the Chesapeake Bay region. Additional information regarding both studies can be found in the appendices section. The appendices include SAS codes used to analyze data (SAS Version 9.2; SAS Institute, Cary, NC), additional graphs and tables depicting all results, maps of the surveyed areas, and photographs of the studies.

**Objectives and Hypotheses**

To begin to understand how varying environmental conditions and invasive species will affect wetlands through the following:

1. To describe aboveground and belowground biomass relationships along estuarine gradient through an observational study.
2. To examine the competitive response between a non-native and a native plant species under different levels of salinity and nutrients in a greenhouse experimental study.

*Observational Study Hypotheses*

- I expect that there will be higher belowground biomass in the oligohaline wetlands along an estuarine gradient on the Nanticoke River.
- The species diversity will be higher in the oligohaline or transitional wetlands than in the fresh or brackish environments.

*Experimental Greenhouse Study Hypotheses*

- *Phragmites australis* will dominate in the lower salinity levels over *Spartina cynosuroides.*
- Higher salinity levels will favor growth of *Spartina cynosuroides* in mixtures containing *Phragmites australis.*
- Higher nutrient levels will assist in the growth of *Phragmites australis* more than *Spartina cynosuroides.*
- Higher salinity levels and low nutrient levels will result in higher stress levels of both species.

# CHAPTER 2

*Observational Study*

**Biomass Allocation of Tidal Wetland Plant Species Along a Salinity Gradient in the Chesapeake Bay: an Observational Study**

## INTRODUCTION

Wetland vegetation is subject to stressors including salinity, inundation, and nutrient availability. The results of these different stressors invoke different responses from the wetland vegetation communities. For instance, increased inundation and salinity are two expected results from sea-level rise that will affect and possibly stress the vegetation communities in wetlands (Spalding and Hester 2007). Naturally the gradual increase of sea level does not create debilitating stress, because wetland environments are able to adapt. Adaptation is seen by the natural increase of elevation which is created by organic inputs like belowground and aboveground biomass (Morris et al. 2002). However with the predicted sea-level rise acceleration in the Mid-Atlantic region (Titus et al. 2009), natural wetland accretion rates may not be able to keep up resulting in wetland loss (Poret-Peterson et al. 2007). Another contributor to subsidence could be from wetlands experiencing higher levels of erosion from larger tidal fluxes (Titus et al. 2009, Mariotti et al. 2010). Erosion is usually ameliorated by vegetation containing sediments with belowground biomass, but with increased inundation wetland plants may have trouble surviving the saturated conditions due to the anoxic conditions (Mendelssohn et al. 1981, Pezeshki 2001). The decrease of belowground biomass levels might be from the increase of nutrients (Valiela et al. 1976, Levine et al. 1998, Morris and Bradley 1999, Turner et al. 2004, Darby and Turner 2008) from anthropogenic and natural sources (Foreman 2009) or from less biomass production due to salinity and

inundation stresses. Thus without the contributions of vegetation communities, marshes can be reduced to open water (Valiela et al. 1976, Delaune et al. 1994).

One of the indicators of marsh collapse to open water is the reduction of belowground biomass. To assess the biomass distribution of a particular region of the Chesapeake Bay watershed, an observational study was conducted on a salinity gradient along the Nanticoke River. Previous observation of the wetlands along the salinity gradient suggest that the oligohaline or transitional zones (0.5-5 ppt) sites may not be keeping pace with sea-level rise in the Chesapeake Bay which may indicate a lack of belowground biomass (unpublished data L. Beckett and J. Allen). Due to the wide range of species present (Sharpe and Baldwin 2009) and their different biomass producing characteristics, the vegetation community could also have an effect on the distribution of biomass. The transitional environments were of interest to see if salinity levels from sea-level rise would have any effect on the distribution of species (Howard and Mendelssohn 1999a, b, 2000). It was hypothesized that the belowground biomass would be high in the transitional wetland environment and lower in the well established brackish and fresh wetland tidal zones due to the higher number of species able to survive in the transitional zones (Sharpe and Baldwin 2009). It was expected that the transitional zones would have higher diversity comprised of both halophytes and fresh water species based on a previous study done by Sharpe and Baldwin (2009).

# METHODS

## Site Description

The study was conducted at five locations along a salinity gradient on the Nanticoke River, a subestuary of the Chesapeake Bay, on the Eastern Shore of Maryland

(Appendix C- 1). Surface elevation tables (SET) were previously set up by Leah Beckett in 2007 at the fifteen sites in five different locations. There were three sites at each location to act as replicates of the conditions at each location. Sites were randomly chosen with respect to location and distance from natural levees which ensured that the sites were located in the interior of the marsh. Sites 1 and 2 were considered brackish wetlands with salinities ranging from 10-21 ppt. Sites 3 and 4 were considered an oligohaline or transitional wetlands with salinities ranging from 2-5 ppt. Lastly, site 5 was characterized as tidal fresh wetlands with low salinities from 0.1-0.5 ppt (unpublished data L. Beckett).

**Biomass Collection**

At each of the 15 sub-sites, five locations each with three replicate sites, three 1-$m^2$ plots were sampled (i, ii, iii) resulting in a total of 45 sample plots. On August 25-27, 2009, aboveground and belowground biomass samples were taken from the sample plots. Aboveground biomass was collected from 1-$m^2$ plots, created with a premeasured PVC quadrat, near the previously established SET site (unpublished data L. Beckett). Sample plots' locations were chosen randomly with respect to the SET site as a general guideline. This allowed for the sample plots to represent the diversity of vegetation at the different locations. All of the aboveground biomass within the sample plot was cut completely to the soil surface, and then placed into large plastic bags for transport. Stems were cut down with serrated knives to ensure that all biomass was collected. Belowground biomass samples were taken at each sample plot after aboveground biomass was collected with a 5cm by 50cm peat corer resulting in half cylinder cores. Each core was then separated into 10 centimeter sections and placed in bags for transport. Three cores

were taken at each plot and the sections were homogenized by depth to provide an adequate description of each sample plot.

All samples were stored in a cold chamber (4°C) until processed to prevent rotting. The aboveground samples were first separated by living or dead samples, and then the live biomass was sorted by species. The belowground biomass samples were rinsed on an 850 µm sieve to separate the soil material from the belowground biomass. All samples were dried at 70°C to a constant weight and then weighed to the nearest 0.01g.

### Statistical Analysis

Analyses were done using a multi-way analysis of variance with a SAS program, PROC MIXED, and the means were separated using Tukey HSD (Saxton 1998) (SAS Version 9.2; SAS Institute, Cary, NC).

## RESULTS

### Belowground Biomass Comparison

The total belowground biomass varied significantly between the sub-sites (Figure 2.1) ($\alpha = 0.05$, $p = 0.0011$). Sites 1 and 2 had mean values ranging from about 15g to about 30g, whereas values from sites 3 and 4 had a larger range of about 10g to about 40g.

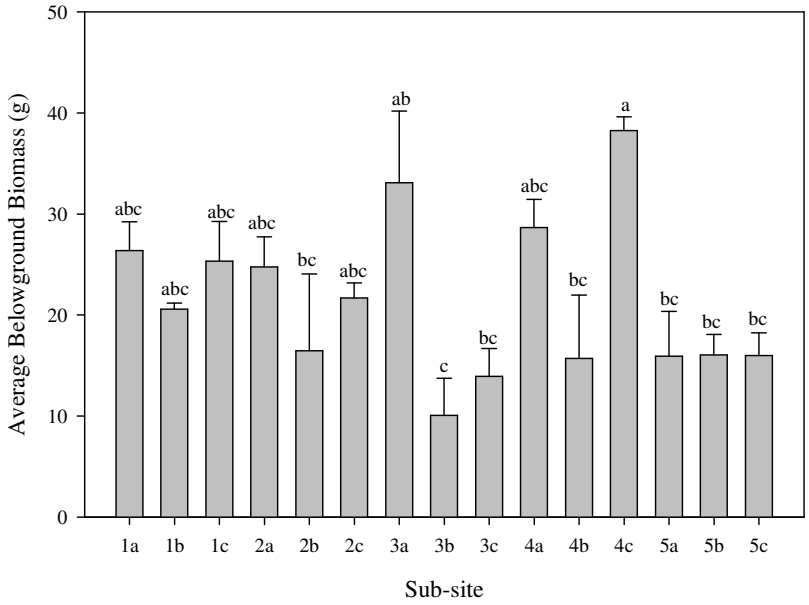



Figure 2.1: Comparison of the belowground biomass from the 3 cores taken equaling an area of 0.00294 $m^2$ at each sample quadrat averaged over each sub-site, showing the high variation between sub-sites. (Salinity decreases from left to right.) Sites 1 and 2 are brackish wetlands, sites 3 and 4 are oligohaline wetlands, and Site 5 is a fresh wetland environment. Values are arithmetic means; error bars are standard error. Different lowercase letters denote significant differences in values ($\alpha = 0.05$).

*Rooting Depth Patterns*

Based on the sub-site location, the three replicates at each site, there was a difference in the rooting depth pattern with respect to the amount of biomass per depth between locations (location x depth $\alpha = 0.05$, $p = 0.0451$) (Figure 2.2 and Table 2.1). Comparative analysis was done between sub-sites due to the high variation seen within the sites (as shown in Figure 2.1). Site 3 had both the lowest and highest value of all sites and depths within the lower 20cm of the core. Additionally, site 3 generally had the lowest biomass at the top 10cm compared to the other sites. Site 5 had the lowest total belowground biomass and the biomass was consistently low throughout the entire 50cm depth profile. Site 1 had the highest top 10 cm values. Site 4 had the highest total belowground biomass of all the sites. The high values of site 4 were seen in the middle

sections (10-40 cm below the soil surface) of sub-sites' 4a and 4c cores. Site 2's values were generally consistent with depth.

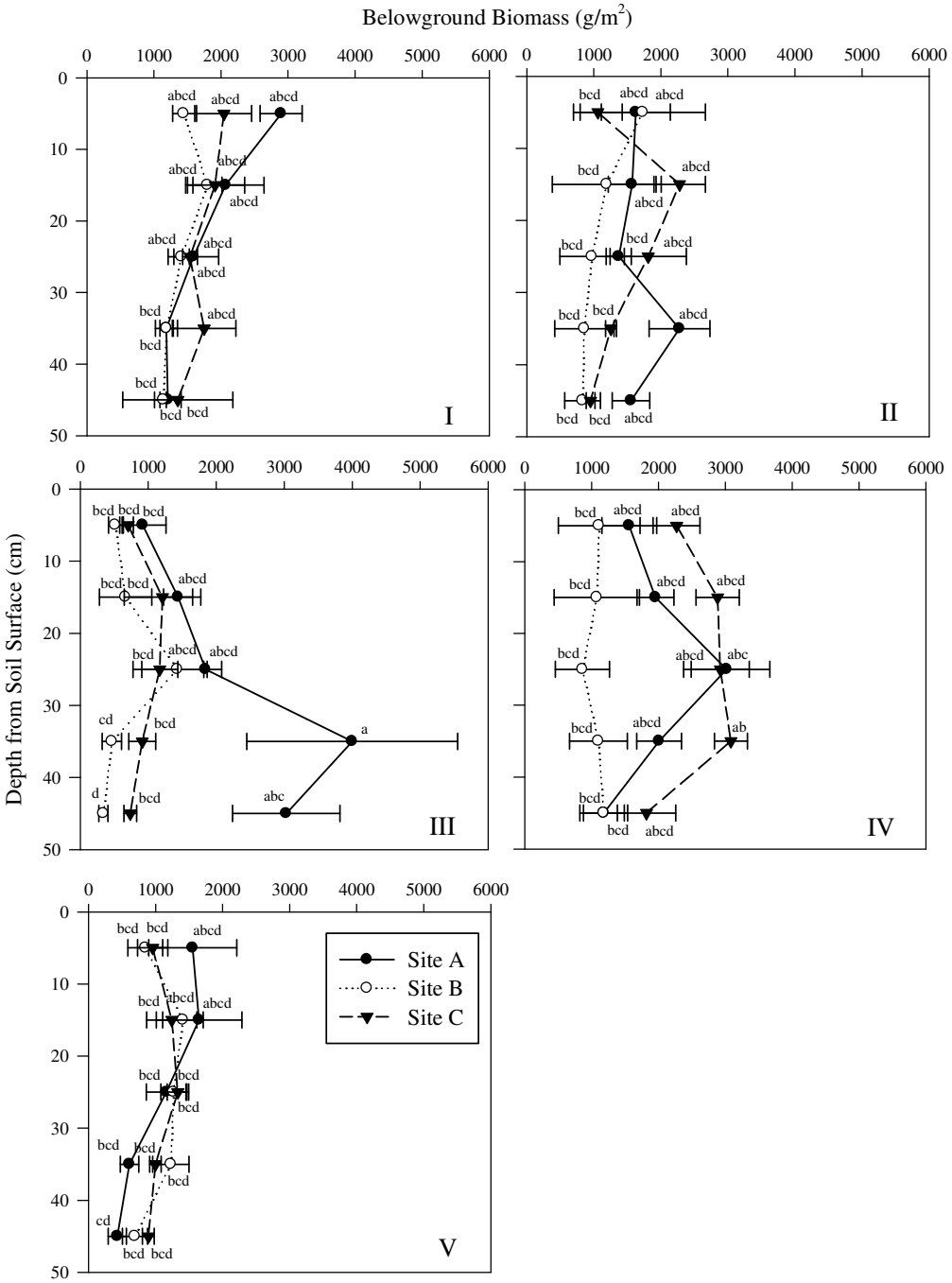



Figure 2.2: Belowground biomass depth distribution based on 10 cm core sections; points represent the data from the entire 10 cm section. I. Site 1, brackish. II. Site 2, brackish. III. Site 3, oligohaline. IV. Site 4, oligohaline. V. Site 5, fresh. Values are calculated estimates based on measurements taken from the 3 cores equaling an area of 0.00294 $m^2$, which has been extrapolated to 1-$m^2$ plots. Values are arithmetic

means; error bars are standard error. Different lowercase letters denote significant differences in values (α = 0.05).

Table 2.1: ANOVA table on depth and sub-site location for belowground biomass rooting depth patterns.

| Source | ndf, ddf | F | P |
|---|---|---|---|
| Sub-site Location | 14, 150 | 7.60 | <0.0001 |
| Depth | 4, 150 | 2.83 | 0.0266 |
| Sub-site Location x Depth | 56, 160 | 1.43 | 0.0451 |

## Aboveground Biomass Comparison

The amount of aboveground biomass was not significantly affected by the location along the salinity gradient and significant differences are only seen at some sub-sites (α = 0.05, p < 0.0001) (Figure 2.3). The highest amount of aboveground biomass collected was at sub-sites 1c and 2c. Site 1c contained a densely populated area of *Juncus roemerianus* Scheele and site 2c had a high percentage of *Spartina cynosuroides* (L.) Roth.

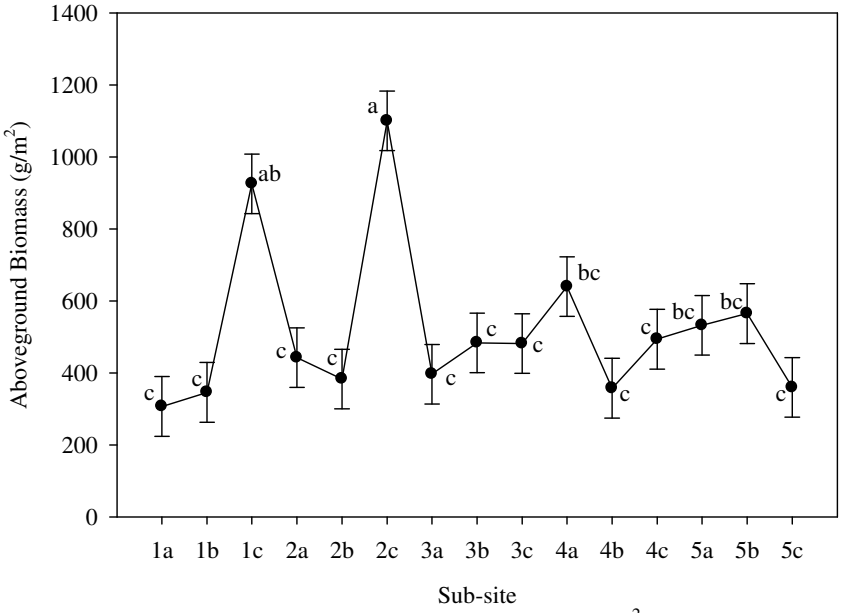



Figure 2.3: Comparison of the aboveground biomass measured in 1-$m^2$ quadrats averaged over each sub-site. (Salinity decreases from left to right.) Sites 1 and 2 are classified as brackish wetlands, sites 3 and 4 are classified as oligohaline wetlands, and Site 5 is a tidal fresh wetland environment. Values are least square means (n=3); error bars are standard error. Different lowercase letters denote significant differences in values (α = 0.05).

**Species Composition**

High variation between the five sites or the fifteen sub-sites was also seen in the biomass of abundant species seen per 1-$m^2$ plot, thus creating large standard error bars (Figure 2.4 and Figure 2.5) ($\alpha = 0.05$, $p < 0.0001$). Abundant species are defined in this study as species that inhabit at least two sites and the majority of biomass measured was greater than 25g. The brackish sites, 1 and 2, were dominated by the following species: *Spartina alterniflora* Loisel., *Spartina patens* (Aiton) Muhl., and *Schoenoplectus americanus* (Pers.) Volkart ex Schinz & R. Keller. Sites 3 and 4, which are classified as oligohaline wetlands, have multiple different species; the highest biomass producing species are *Polygonum arifolium* L.*, Symphyotrichum puniceum* (L.) A. Löve & D. Löve*,* and *Typha latifolia* L.*.* The highest biomass producer at Site 5, the fresh wetland environment, was *T. latifolia* with contributions from *Impatiens capensis* Meerb.*,* *Peltandra virginica* (L.) Schott*, P. arifolium,* and *S. puniceum.*

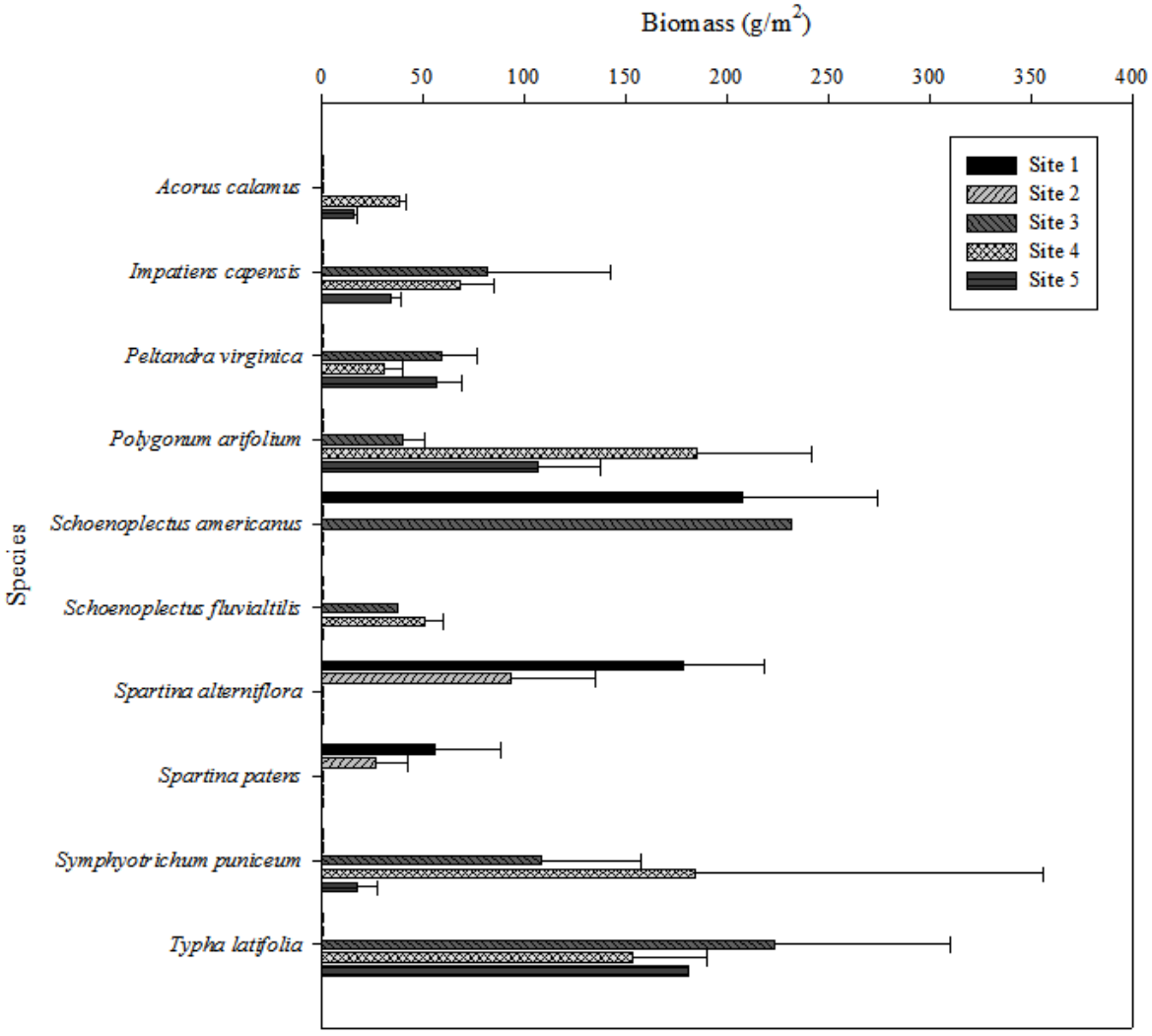



Figure 2.4: Comparison of aboveground biomass of the ten most abundant species. Abundant species are defined as species present in at least two sites and contain biomass measurements greater than 25g in most plots. Salinity decreases from site 1 to site 5. Values are arithmetic means and error bars are standard error.

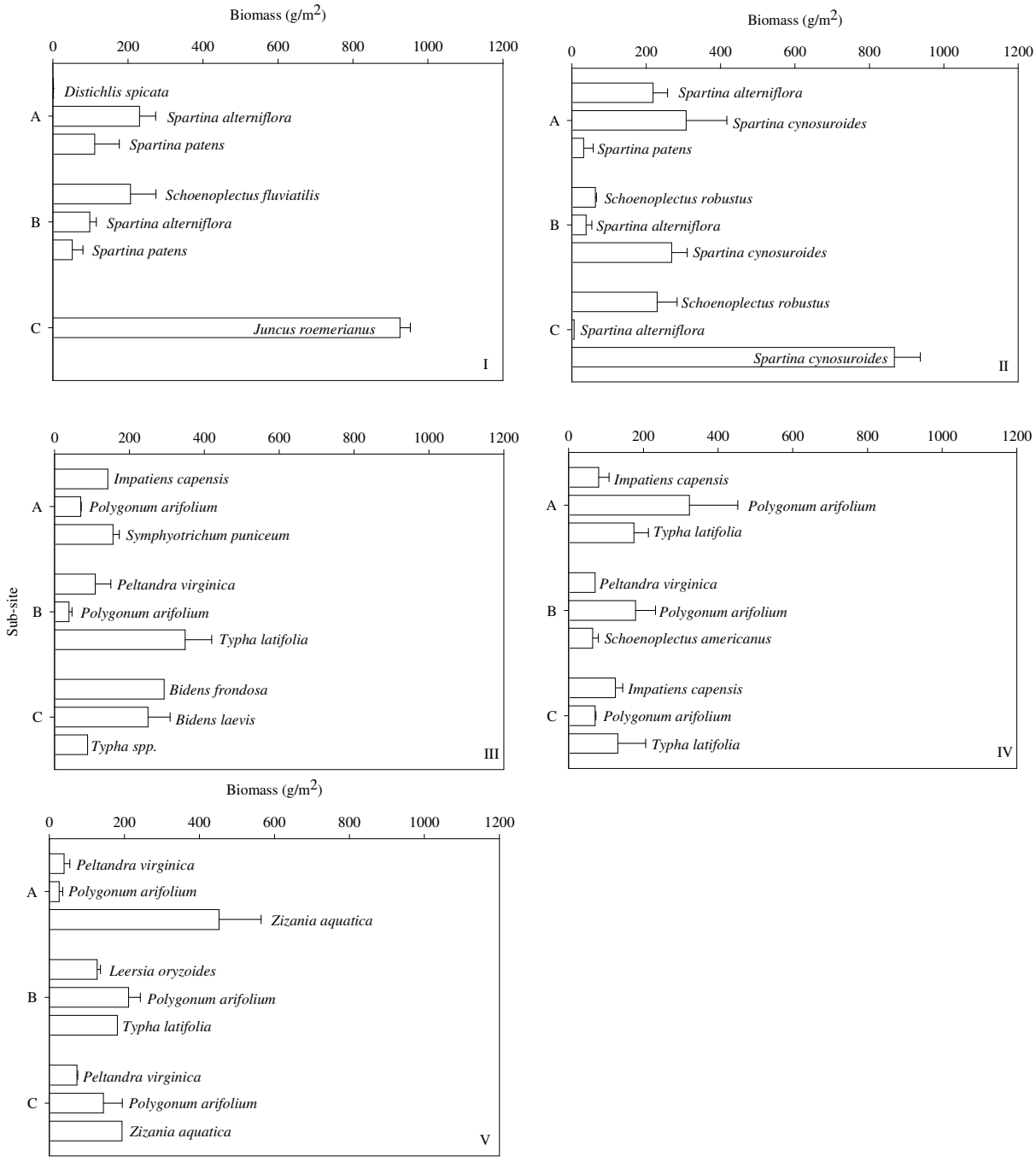



Figure 2.5: Comparison of aboveground biomass of each site by sub-site showing the three highest producing species in each sub-site. I. Site 1, brackish. II. Site 2, brackish. III. Site 3, oligohaline. IV. Site 4, oligohaline. V. Site 5, fresh. Values are arithmetic means and error bars are standard error.

The number of species per sample plot varied between sub-sites, which is seen by the large standard error bars (Figure 2.6) ($\alpha = 0.05$, $p < 0.0001$). The lowest diversity plots were seen in the brackish environments, especially site 1c where the only species present was *J. roemerianus* (Figure 2.5). The highest values were seen at the oligohaline wetland in site 4, specifically 4c. There was only a significant difference at the 0.10 level between site 1 and 4 (Figure 2.7) ($p = 0.0949$).

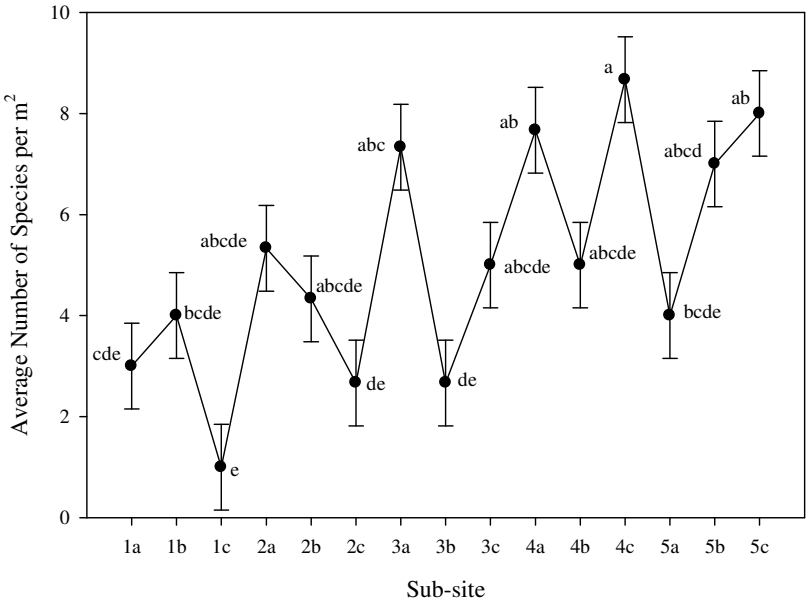



Figure 2.6: Comparison of the number of different species in each square meter sample quadrat for each sub-site showing the large variation between sub-sites. (Salinity decreases from left to right.) Sites 1 and 2 are brackish wetlands, sites 3 and 4 are oligohaline wetlands, and Site 5 is a fresh wetland environment. Values are least square means; error bars are standard error. Different lowercase letters denote significant differences in values ($\alpha = 0.05$).

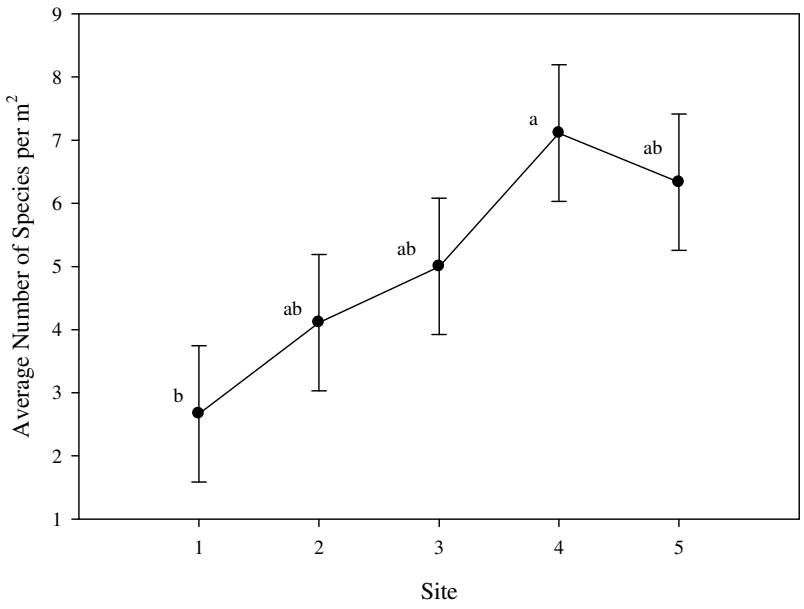



Figure 2.7: Comparison of the number of different species in each square meter sample plot for each site showing the high variation between sites. (Salinity decreases from left to right.) Sites 1 and 2 are brackish wetlands, sites 3 and 4 are oligohaline wetlands, and Site 5 is a fresh wetland environment. Values are least square means; error bars are standard error. Different lowercase letters denote significant differences in values ($\alpha = 0.10$).

Comparisons of the amount of biomass, including aboveground, belowground, and total, to the number of different species within each site showed no significant relationships (Figure 2.8).

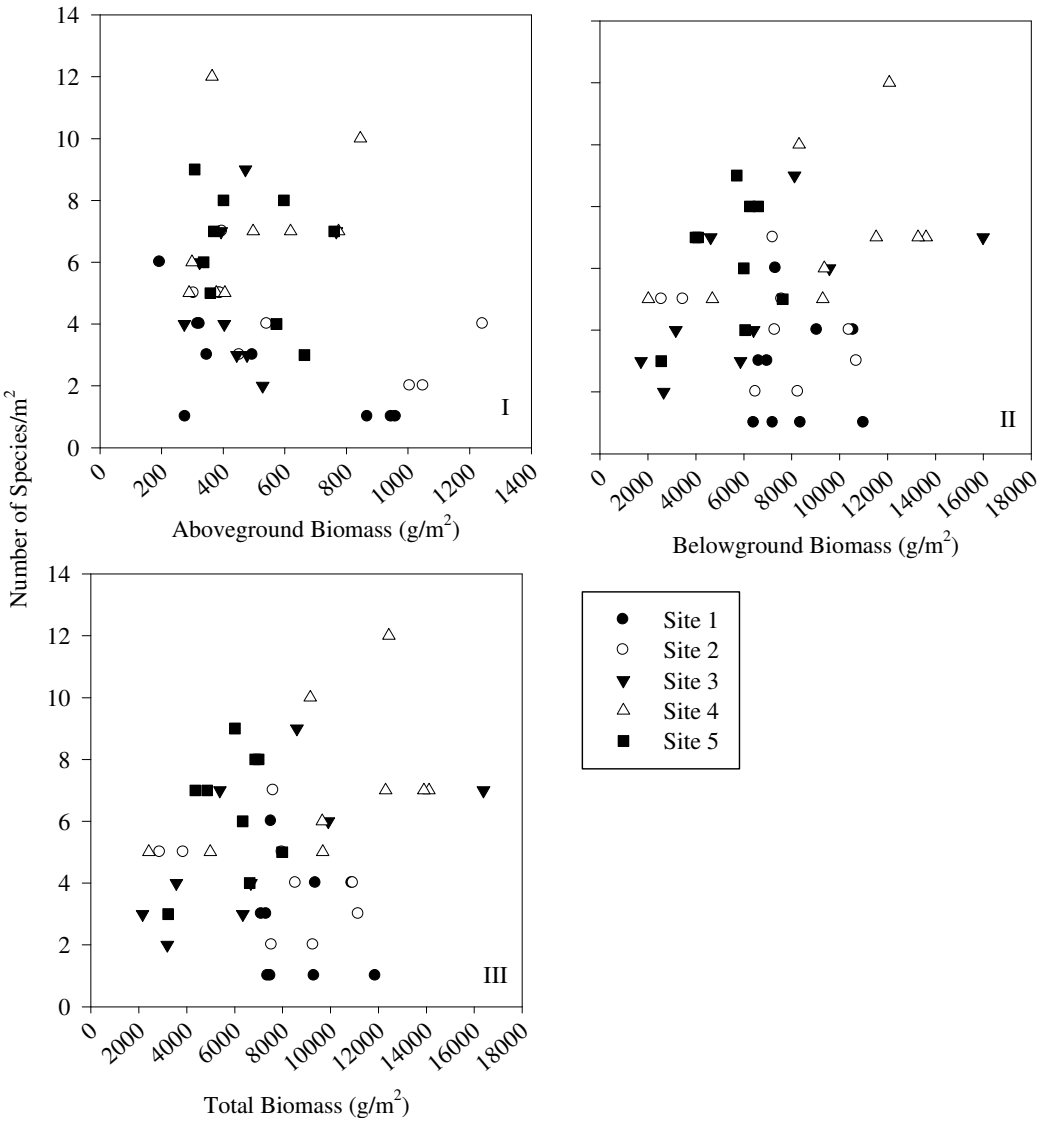



Figure 2.8: Comparison of the number of different species in each square meter sample plot for each site and biomass. I. Aboveground biomass per $m^2$ II. Belowground biomass per $m^2$ III. Total biomass per $m^2$ (Salinity decreases from left to right.) Sites 1 and 2 are brackish wetlands, sites 3 and 4 are oligohaline wetlands, and Site 5 is a fresh wetland environment. Each point represents the data from each of the 45 sample quadrats.

## DISCUSSION

The evaluation of these sites was completed in order to examine the allocation of biomass along a salinity gradient and biomass relationships with respect to species diversity. The high belowground biomass values could be attributed to the lack of separation of live and dead belowground biomass material; other studies separated live

and dead belowground biomass material and found lower values (Turner et al. 2004, Darby and Turner 2008). Additionally values could be skewed based on different rooting patterns of species, so tightly bound or dense root systems might not have been appropriately sampled. There were differences in the distribution and amount of biomass by salinity and species diversity. This could have implications for wetland management, especially with regards to conservation and restoration efforts, by knowing biomass habits managers could create more stable wetland environments.

**Oligohaline Wetland Sites**

As mentioned previously, oligohaline wetlands along this estuary may not be keeping pace with rising sea level; this could be due to low amounts of belowground biomass contributing to the organic carbon in the substrate relative to other sites. There was low belowground biomass at the 0-30cm levels below the soil surface, which could explain why there is a lack of additions and retention as similarly explained in Hartig et al. (2002) and Darby and Turner (2008). However, the lower values do not correspond to any higher values seen in aboveground biomass in these sites suggesting that there was not a shift similar to the results seen in Valiela et al. (1976) study. The low belowground biomass values could be from the species that were present had wide spreading rhizomatous systems, like *Typha sp.* (Stevens 2006) or tight bulb root systems, like *P. virginica* (USDA 2002) that could have been missed when the belowground biomass samples were randomly taken. Unlike site 3, site 4 had the highest belowground biomass values of all of the sites and the values were consistent with depth. Additionally, site 3a had the highest average number of species per plot and the rest of site 3 and 4 had similarly high diversity. Together with the high belowground biomass values, this could

indicate that there is facilitation or complementarity of resources between the species at site 4, which would result in high biomass production as suggested by other studies (Bertness and Ellison 1987, Bertness 1991, Bertness and Shumway 1993, Bertness and Callaway 1994, Hooper 1998). Since site 4 has average aboveground biomass and high belowground biomass; this may also indicate that the site is nutrient lacking and the species must delve deeper in the soil to find adequate nutrients for growth (Gedroc et al. 1996, Darby and Turner 2008).

**Fresh Wetland Site**

In addition to site 3, site 5 had low belowground biomass values throughout the entire 50 cm depth sampled, and the lowest belowground biomass values of all sites sampled. The low belowground biomass values were consistent across all three sub-sites (Figure 2.1). These low values could indicate a highly nutrient rich environment or different species morphologies, where the rooting systems do not have to search deeper for nutrients as described in Gedroc et al. (1996), or it could indicate a highly competitive environments as suggested in Crain et al. (2004) who had similar results. When examining the aboveground components of this site, the values for biomass are similar to the other sites along the salinity gradient and site 5 had the second highest number of species per plot. The high diversity could be attributed to the low stress environment which allows for competition to dominate in structuring the plant communities (Pennings and Callaway 1992, Gough et al. 1994, Crain et al. 2004, Pennings et al. 2005, Sharpe and Baldwin 2009, Engels and Jensen 2010). Similar studies have shown a similar relationship between high diversity values and low biomass production due to high competition levels (Almufti et al. 1977, Gough et al. 1994).

**Brackish Wetland Sites**

Sites 1 and 2, specifically sites 2c and 1c, had the highest aboveground biomass on the salinity gradient which is similar to the results in Gough et al.'s study (1994). The high biomass values can be attributed to the high biomass producing species, *S. cynosuroides* and *J. Roemerianus* seen respectively at 2c and 1c. The belowground biomass at these sites was fairly consistent with depth and had mid-range values for their total belowground biomass which corresponds with results found in the study conducted by Crain et al. (2004). The species diversity was the lowest at these two sites compared to the other sites along the salinity gradient. This was expected due to the high stress environment of the higher salinity conditions which prevents many species from inhabiting these areas. Species composition is dictated by the species' ability to survive stress-inducing conditions and not competition in the brackish environments as suggested in other studies (Pennings and Callaway 1992, Gough et al. 1994, Crain et al. 2004, Pennings et al. 2005, Sharpe and Baldwin 2009, Engels and Jensen 2010).

**Species Composition**

A widely discussed paradigm is that the high stress environments', like ones that are frequently flooded and have high salinity, composition is dependent upon stress-tolerance and not competition as mentioned previously. In the more favorable conditions, species composition is decided upon competition between species. The relationship of biomass and species richness can be attributed to this paradigm as well. Other studies have shown that there could be a weak inverse relationship between richness and the amount of biomass (Almufti et al. 1977, Gough et al. 1994), which is seen in the aboveground biomass distribution of this study. However, the relationship is likely more

influenced by environmental conditions, including nutrient availability, water resources, and light availability, which would dictate the amount of biomass produced and the competition hierarchy (Gough et al. 1994). There is also a trend within the distribution of belowground biomass and diversity seen in this study; there is more belowground biomass in the highly competitive areas of high species diversity and low biomass in the less diversity communities. This trend corresponds to the findings in root dynamic studies with respect to responses to nutrient availability (Gedroc et al. 1996). The belowground biomass trend corresponds to the diversity paradigm because there would be more competition dictating species growth including root systems in more favorable conditions leading to more roots. However in more stressing environments, energy is allocated to survival through adaptations rather than expansion. This observational study supports the diversity paradigm of community species distribution. Also, adding information about belowground biomass growth could add to our understanding of vegetation dynamics. Future studies could focus on biomass allocation dynamics, especially with respect to evaluating wetlands' persistence in the face of sea-level rise.

# CHAPTER 3

*Experimental Greenhouse Study*

**Understanding Factors of Biomass Allocation: a Competition Study between Non-Native *Phragmites australis* and Native *Spartina cynosuroides***

## INTRODUCTION

One of the factors threatening diverse coastal wetland environments is marsh collapse or 'break-up', which can be caused by various factors including increased inundation, vegetation dieback, and increased nutrients. Marsh collapse occurs when the vegetation cannot survive in the wetland due to increased flooding and the area turns into open water. Invasive species can threaten the composition of the native vegetation by rapidly taking over in disturbed and often undisturbed environments. These invasive species may out-compete native species and create monoculture stands, which often modify the environment that they inhabit. Although these two factors are seen as negative impacts on wetland ecosystems, invasive species could help combat complete marsh die-back and preserve the wetland environments. Invasive species often modify high stress environments to make them more conducive for their growth, thus making them perfect candidates for disturbed wetland environments (Bart and Hartman 2003, Burdick and Konisky 2003). Understanding how different species will react in these different environments will allow managers to create effective practices to sustain these unique ecosystems.

Excess nutrients are added to wetland environments from a variety of anthropogenic sources including agricultural inputs and urban outflows. Specifically from October 2008-September 2009, there was an estimated 240 million pounds of nitrogen going into the Chesapeake Bay from a plethora of sources along its tributaries

including, but not limited to: agricultural runoff, automobile pollution, urban runoff, industrial emissions, and treated wastewater (Foreman 2009). Increase in fertilization, like nitrogen, may cause plants to shift from production of belowground biomass to aboveground biomass thus decreasing inputs of organic matter and decreasing retention capabilities of the soil (Valiela et al. 1976, Minchinton and Bertness 2003, Turner et al. 2004). By reducing belowground biomass, the structure of the wetland soils may be compromised, which can lead to more inundation and eventually marsh collapse (Mendelssohn et al. 1981).

Another factor assisting in the reduction of wetland stability is increased flooding from sea-level rise and other anthropogenic sources. The sea level is higher than it has been since 1870 and is continuing to rise up our shorelines (Titus et al. 2009). Higher water levels could lead to marsh collapse from the inability of wetland plants to survive the saturated anoxic conditions (Pezeshki 2001, Morris et al. 2002). The increase in sea level is also expected to cause higher salinity levels in the fresh (0-0.5 ppt) and oligohaline (0.5-5 ppt) tidal wetlands. The combination of increased flooding and salinity created by sea-level rise produces a highly stressful growing environment that can lower biomass production (Spalding and Hester 2007). Without any vegetation to add new biomass material, the wetlands could subside or die-back due to the low concentration of organic carbon into the soil and the lack of stability for erosion control (Hartig et al. 2002, Morris et al. 2002, Poret-Peterson et al. 2007).

The stressful environments of disturbed wetlands are likely locations of invasion of non-native species like *Phragmites australis* (Cav.) Trin. ex Steud. (Rice et al. 2000, Burdick and Konisky 2003, Alvarez-Rogel et al. 2007, Chambers et al. 2008). Once *P.*

*australis* becomes established, it often alters its surrounding environment by increasing accretion rates of the soil and modifying nutrient cycling (Bart and Hartman 2003, Rooth et al. 2003). Both of these modifications could reverse the negative effects of high nutrient inputs and help prevent marsh collapse (Spalding and Hester 2007). To overcome the stressful environments caused by flooding and higher salinities, *P. australis* uses long tillers (up to 10 m) and deep root systems (up to depths of 60 cm) to establish connections to areas with more favorable conditions including ones with more nutrients and/or lower salinity levels (Adams and Bate 1999, Bart and Hartman 2002, 2003). Additionally these root systems are able to transmit oxygen from oxic conditions to saturated conditions to allow for expansion into unfavorable anoxic environments (Brix et al. 1996). The addition of fertilization also assists *P. australis* in surviving disturbed and stressful growing conditions (Rickey and Anderson 2004). By being able to survive in flooded, saline conditions, *P. australis* may be a valuable asset in preventing marsh collapse.

To assess if the conditions of sea-level rise and high nitrogen pollution will create an environment where *P. australis* is dominant over the native species, a greenhouse competition experiment was conducted between the native species, *Spartina cynosuroides* (L.) Roth, and the non-native *P. australis*. These species were chosen due to their similar morphological characteristics (both species are tall, clonal perennial grasses) and their coexistence in similar marsh environments. It has been found that these species coexist in the transitional wetland zones of both the Nanticoke and Patuxent Rivers in the Chesapeake watershed (Sharpe and Baldwin 2009). *Spartina cynosuroides* is a native Chesapeake Bay species in fresh and brackish tidal marshes. It is a dominant

species in the Chesapeake Bay area, and it creates detritus that is valuable to the wetland ecosystem. This detritus is often used by muskrats, geese, and other wetland species for food and habitat construction (Silberhorn 1992). Due to the high production of biomass, it is said to be one of the most important tidal wetland species (Silberhorn 1992).

The study was conducted to observe the competitive interactions between the two species, *P. australis* and *S. cynosuroides*, in fresh and oligohaline wetland environments. The study was to visualize the characteristics of *S. cynosuroides* and *P. australis* in any of the proposed simulated environments with regards to biomass allocation dynamics. I hypothesized that the *S. cynosuroides* would dominate in high salinity environments over *P. australis* and the opposite would occur in low salinity environments. However, in high nitrogen environments, I predicted that *P. australis* would have an advantage over *S. cynosuroides*, because the nitrogen addition could alleviate the stress caused by high salinity levels (Levine et al. 1998, Emery et al. 2001).

# METHODS

## Mesocosm Construction

To measure the effect between *P. australis* and *S. cynosuroides* in varying levels of nutrients, competition, and salinity, seventy-two mesocosms were constructed and maintained in the Research Greenhouse on University of Maryland College Park campus. The mesocosms were constructed out of 13 gallon HDPE (high density polyethylene) rectangular pails (16.25" L x 13.25"W x 19"H) containing growing medium (Ewing 1996, Howard and Mendelssohn 1999a, Lissner et al. 1999). The growing medium was created by combining three parts professional potting mix LC-1 (Sungro Horticulture, Bellevue, WA) and one part fine grade sand. The potting mix contains Canadian

sphagnum peat moss, coarse grade perlite, gypsum, and dolomitic lime. After initial overnight saturation, the tested pH of the growing medium was around 6.1. This medium was used to ensure the complete removal of belowground biomass at harvest without additional previous material. Each mesocosm initially contained six stems of either: all *P. australis*, all *S. cynosuroides,* or three stems of each species, creating a replacement competition design (Gibson et al. 1999, Jolliffe 2000, Connolly et al. 2001). To mimic the typical flooding regime of tidal coastal high marsh communities of 30-35% observed flooding (Mitsch and Gosselink 1993), each mesocosm was flooded for 2.1 days each week. This value is derived from the 30% observed flooding time over an entire week period, resulting in 2.1 days flooded in a 7 day time span. Flooding levels were maintained at 10 cm above the soil surface, and non-flooded levels were kept at 10 cm below the soil surface. The systems were completely flushed fortnightly to ensure no excess buildup of salts or nutrients. In addition to the flooding, drip irrigation was installed for each mesocosm with tap water to replenish water lost via evaporation and absorption, approximately two gallons a day. The appropriate water levels were controlled through a regulation system containing a drain at the bottom of each system with a tube to ensure proper flooding levels (Figure 3.9).

Figure 3.9: The construction of all 72 mesocosms which allows for proper drainage, complete belowground biomass removal, and water level of each mesocosm independently.

The bottom drain was covered by a 1x2 mm screen and enveloped by river rocks with a layer of weed prevention material on top to allow for complete drainage. When completely flooded, the water levels were regulated by a drainage hole at the calculated flood water level. Additional growing medium was added half way through the experimental time period due to material loss from drainage and compaction. After a month of growth, 40% white shade cloths (Gempler's, Madison, WI) were installed around each mesocosm to increase isolation of mesocosms and prevent plants from falling over. Shade cloths also mimicked field conditions by partially shading the sides of the plants as seen in dense stands in the field.

**Treatment Application**

Each solution of the proper salinity and nutrient level was created individually for each mesocosm to ensure independence between each of the mesocosms (personal communication with B. Momen). Salinity pulse levels were achieved with Instant Ocean synthetic sea salt to create four different levels of salinity (0, 4, 8, and 16 ppt) (Aquarium Systems, Inc. Mentor, OH). These values are based on the historical maximum,

previously observed maximum, previously observed mid-range value, and a control value in similar locations along the Nanticoke and Patuxent Rivers (Sharpe and Baldwin 2009). Each salinity regime was created using premeasured containers of Instant Ocean in mixing buckets, and the appropriate amount of tap water was added to each. Eleven liters and thirteen liters were alternated weekly; thirteen liters was used to completely flush the mesocosms. The high nitrogen treatment was based on a loading rate of 300 g $N/m^2yr$ and achieved with granular urea (46/0/0) nitrogen fertilizer (Southern States Cooperative, Inc., Richmond, VA). This loading rate was based on a mid-range value of a literature review of similar studies; the range of values went from 12 g $N/m^2yr$ to about 500 g $N/m^2yr$ (Morris 1991, Mitsch and Gosselink 1993, Levine et al. 1998, Crain 2007, Brin et al. 2010). The low nitrogen treatment did not have any nitrogen additions aside from the small initial nutrient charge contained in the growing medium and from mineralization of organic matter in the growing medium. Nitrogen additions of 1.74 g of granular urea were sprinkled on the soil surface each week before the salinity treatment. The experiment was conducted for six months.

**Experimental Design**

The experiment was a 3 x 4 x 2 complete factorial randomized block design resulting in 24 different treatments comprised of 3 competition levels, 4 salinity levels, and 2 nitrogen levels. There were three blocks acting as replicates, and each block contained each treatment once. The assignment of treatments for each mesocosm was completely randomized.

**Sample Collection**

Rhizomes and small shoots of each species were collected at wetlands adjacent to the Clyde Watson Boating Launch on the Patuxent River in Brandywine, Maryland. In this oligohaline wetland, both species were coexisting at similar elevations. Rhizomes and shoots of *P. australis* and *S. cynosuroides* were collected in March and April 2010. The samples were then placed in growing medium in flat trays and pots. The samples were kept moist and fertilized in the misting room of the Research Greenhouse until they were ready for transplanting in late May 2010.

**Planting**

Approximately six stems were planted in each mesocosm in late May. Stems were selected so that each mesocosm would have similar initial biomass. This was done by counting smaller clonal ramets as half of a stem and any shoots smaller than 3 cm were not counted in the biomass calculations. The rhizomes plus 1-cm of the stem were planted beneath the soil surface. Mixed species mesocosms contained three stems of each species with similar initial biomass of each species. The different species were initially planted at opposite ends of the mesocosm. All mesocosms were allowed to acclimate for a week before treatments were applied.

**Nondestructive Measurements**

Plant morphological measurements were made during the growing season to assess growth dynamic parameters and to quantify stress levels. Morphological assessments were taken by surveying stem height and number of stems in each mesocosm in June, July and September 2010. Stem heights were also collected during harvesting in December 2010. These surveys were used to calculate relative growth rates (RGR) per

day over the entire six month growing period, which was estimated using two different methods: overall RGR and average RGR. Average RGR calculated each of the three rates between the four stem height measurements and then averaged them together. Overall RGR was calculated by using the measurements taken in June and December and calculating the RGR between them using the whole six month time period. Early RGR was calculated between June-July, July-September, and June-September to depict the growing patterns throughout the experiment and to check if there were any effects of possible pot-bounding. A biomass extrapolation completed by running regression analysis to determine a relationship between stem height and aboveground biomass. This relationship was then used to extrapolate the aboveground biomass in September based on the stem heights measured. This analysis was done see if the results in September were similar to the final results seen in December. Further extrapolation was not done for the stem height measurements in June and July due to the high variability and possible errors.

Additionally during the growing season, fluorescence was measured using the WALZ PAM-2100 portable chlorophyll fluorometer (Walz, Effeltrich, Germany) to detect possibly photosynthetic stress. Measurements of $F_v/F_m$ (leaf fluorescence) were taken from the third leaf from the shoot apex (Adams and Bate 1999) which were not dark adapted. When light energy is absorbed on a leaf, only some of the energy can be used for the process of photosynthesis and the remaining energy is released as heat or light energy. This excess light energy is known as fluorescence and can be quantified with a chlorophyll fluorometer. The amount released gives an estimate on how much of

the light is being used for photosynthesis, which can be used to assess the stress experienced by the plant (Maxwell and Johnson 2000).

To assess the salinity treatments, a two week water sub-sampling event was conducted on only mixed species mesocosms. The salinity readings were taken from the outflow water from the regulator tubes and surface water when available with an YSI model 30 salinity meter (YSI, Yellow Springs, Ohio).

**Aphids**

During the six month growing period, there were observations of aphid infestation on multiple mesocosms on both species. To eradicate the aphids, pesticides were sprayed in late September 2010 with a mix of Talstar (3 mL/gal) (FMC Corporation, Philadelphia, PA), Ovation (1 mL/gal) (Scotts-Sierra Crop Protection Company, Marysville, OH), and Enstar II (2 mL/gal) (Wellmark International, Schaumburg, IL). The pesticides controlled the infestation but did not eradicate the aphids entirely. In early December, to prevent further damage, 1,500 ladybugs, 500 ladybugs on each block, were released in efforts to further remove the aphids. The ladybugs had little effect on the population of aphids, but no further action was needed before harvesting.

**Destructive Measurements**

Complete harvesting of all of the mesocosms was done in late December 2010. To determine the effects of competition, fertilization, and salinity within the mesocosms, biomass measurements were taken and then compared between species. All aboveground biomass was cut at the soil surface and then stem heights were measured. The aboveground biomass was then placed in paper bags for drying and weighing.

Belowground biomass collection procedures varied between the monoculture mesocosms and the mixed species mesocosms. The belowground biomass in mixed mesocosms was separated by species only and not by depth. This was done by splitting each mesocosm down the original planting division line and following the shoots of the individual plants to its roots. Often there were coloration and morphological characteristics that could identify the species' belowground biomass apart, but if there was no way of distinguishing the sample, it was placed in an unknown species sample bag for each mesocosm. These unknown samples were added into the full belowground biomass amounts and were not included in the individual species biomass calculations. In the monoculture mesocosms, the soil was split into 10-cm horizons and the biomass was collected by depth. This was done to compare the rooting depth patterns between the two species as well as to compare how the treatments affected rooting depth patterns. Once collected, all belowground biomass was washed over a 1x2-mm mesh screen then placed in paper bags to be dried to ensure that all soil material was removed from the biomass (Megonigal and Day 1992). All biomass was dried to a constant weight in an environmental chamber at 35.2 °C with 12% humidity for at least 12 days. To compare monoculture mesocosms' biomass to mixed species mesocosms, the values were adjusted by dividing by two to account for the differences in biomass at the initial planting.

**Statistical Analysis**

Analyses were done using a multi-way analysis of variance with a SAS program, PROC MIXED, and the means were separated using Tukey HSD (Saxton 1998) (SAS Version 9.2; SAS Institute, Cary, NC). Regression analysis was done using PROC REG in the SAS program.

# RESULTS

## Salinity

The salinity levels within each mesocosm fluctuated over the week treatment cycle within the outflow and the surface water (Table 3.2).

Table 3.2: Summary of the four salinity regimes over the two week treatment cycle on mixed mesocosms.

| Treatment | | Outflow Salinity | | | Surface Water Salinity | | |
|---|---|---|---|---|---|---|---|
| Nitrogen Level | Salinity Regime | Average ± SE (ppt) | Absolute Range (ppt) | Average Range (ppt) | Average ± SE (ppt) | Absolute Range (ppt) | Average Range (ppt) |
| No Nitrogen | A | 0.22 ± 0.02 | 0.10-0.50 | 0.11-0.36 | 0.22 ± 0.02 | 0.10-0.80 | 0.13-0.32 |
| | B | 1.03 ± 0.02 | 0.10-1.90 | 0.86-1.17 | 2.38 ± 0.07 | 0.80-4.20 | 2.12-2.67 |
| | C | 1.85 ± 0.04 | 0.40-3.70 | 1.62-2.16 | 4.26 ± 0.06 | 1.10-8.10 | 4.00-4.45 |
| | D | 3.91 ± 0.13 | 0.90-9.60 | 3.17-4.81 | 7.75 ± 0.13 | 1.40-16.10 | 7.22-8.27 |
| Nitrogen | A | 0.34 ± 0.04 | 0.10-0.90 | 0.18-0.61 | 0.29 ± 0.06 | 0.10-1.10 | 0.13-0.58 |
| | B | 1.40 ± 0.05 | 0.40-3.20 | 1.13-1.69 | 2.16 ± 0.10 | 0.20-4.10 | 1.72-2.58 |
| | C | 2.12 ± 0.10 | 0.60-5.00 | 1.58-2.77 | 4.02 ± 0.16 | 0.60-8.00 | 3.37-4.68 |
| | D | 4.08 ± 0.15 | 0.80-8.80 | 3.20-5.03 | 6.83 ± 0.28 | 0.20-15.80 | 5.50-7.67 |

Values are average ± SE. Salinities were measured on mixed species mesocosms only. Outflow salinity was measured daily over a two week watering cycle, which started with complete flush of the mesocosms and then a replenish charge for a total of 14 days. Outflow water was collected from the regulator tube after the standing water contents of the tube had been drained out completely. Both the outflow salinity and surface water salinity was measured with an YSI model 30 salinity meter (YSI, Yellow Springs, Ohio). Surface water salinity was measured for 6 days of the 14 day cycle when the mesocosms were flooded. Salinities were achieved by adding salt water solution of 0,4,8, and 16 ppt weekly with a 2.1 day flooded period and daily irrigation to replenish absorbed and evaporated water. These four levels are referred to as A, B, C, and D, where A is the lowest salinity (created with 0 ppt) and D is the highest salinity (created with 16 ppt). Differences between salinity levels was significant at the alpha equals 0.05 level. There were no significant differences of the nitrogen treatment on the salinity levels.

The levels would peak in the first two days of treatment application and decrease through the remaining week for both outflow (Figure 3.10) and surface water (Figure 3.11). The outflow water salinity was lower than the surface water salinity, which is similar a study done by Adams & Bate (1999) where their interstitial water had a lower salinity than their surface water.

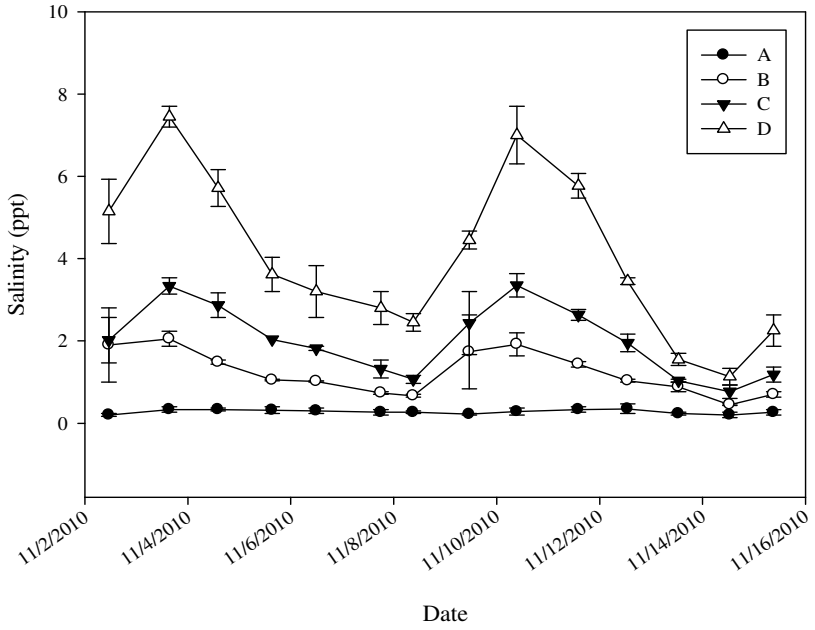



Figure 3.10: Outflow water salinity levels over a two week sampling period of only mixed species mesocosms. Salinity levels increase from A to D. The values represented are arithmetic means averaged over nitrogen levels. Error bars are standard error.

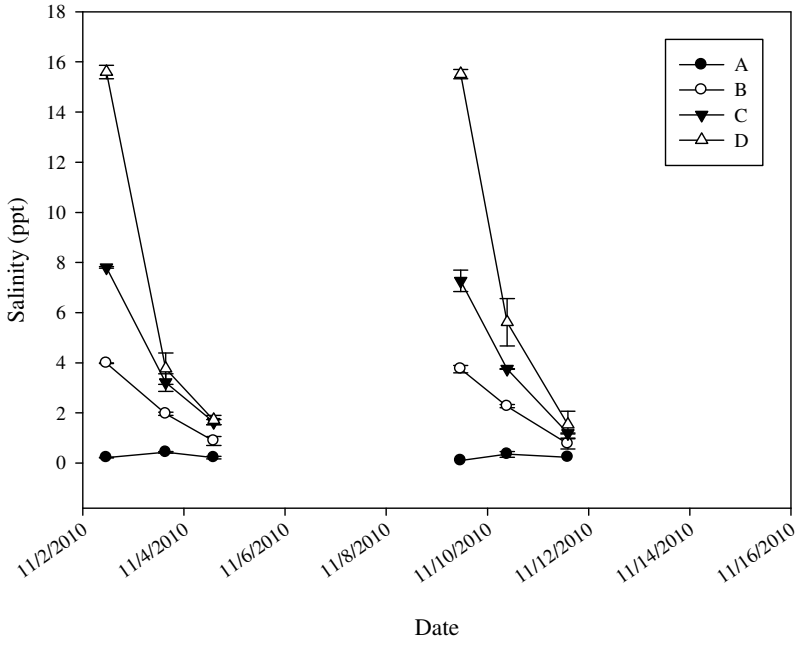



Figure 3.11: Surface water salinity levels over a two week sampling period consisting of mixed mesocosms. Values represented are arithmetic means averaged over nitrogen levels. Salinity levels increase from A to D. Error bars are standard error.

The average salinities of each treatment level were found to be statistically different from each other ($\alpha = 0.05$, $p < 0.0001$).

## Relative Growth Rates

*Overall RGR*

Fertilization significantly increased *P. australis* growth compared to unfertilized mesocosms ($\alpha = 0.05$, $p < 0.0001$). RGR of *P. australis* was also positively affected by the competition from *S. cynosuroides* by an average difference of 0.00206 cm $cm^{-1}$ $day^{-1}$ from growth in monoculture mesocosms ($\alpha = 0.05$, $p < 0.0001$) (Table 3.3, see appendix E-1).

Table 3.3: ANOVA summary for *P. australis*' overall RGR, which was calculated from stem height measurements taken in June and December 2010 for all treatments.

| Source | ndf, ddf | F | p |
|---|---|---|---|
| Salinity Regime | 3, 30 | 0.57 | 0.6416 |
| Nitrogen | 1, 30 | 343.34 | **<0.0001** |
| Competition | 1, 30 | 29.93 | **<0.0001** |
| Salinity Regime x Nitrogen | 3, 30 | 0.96 | 0.4234 |
| Salinity Regime x Competition | 3, 30 | 0.34 | 0.7966 |
| Nitrogen x Competition | 1, 30 | 0.05 | 0.8319 |
| Salinity Regime x Nitrogen x Competition | 3, 30 | 0.30 | 0.8253 |

Bolded values indicate significance at $\alpha = 0.05$.

In the fertilized mesocosms, the monocultures of *S. cynosuroides* had a significantly higher RGR than the stems within the mixed mesocosms with *P. australis.* However, in the unfertilized mesocosms, there was no significant difference between mixture and monoculture mesocosms, resulting in a significant interaction ($\alpha = 0.05$, $p = 0.0082$) (Figure 3.12).

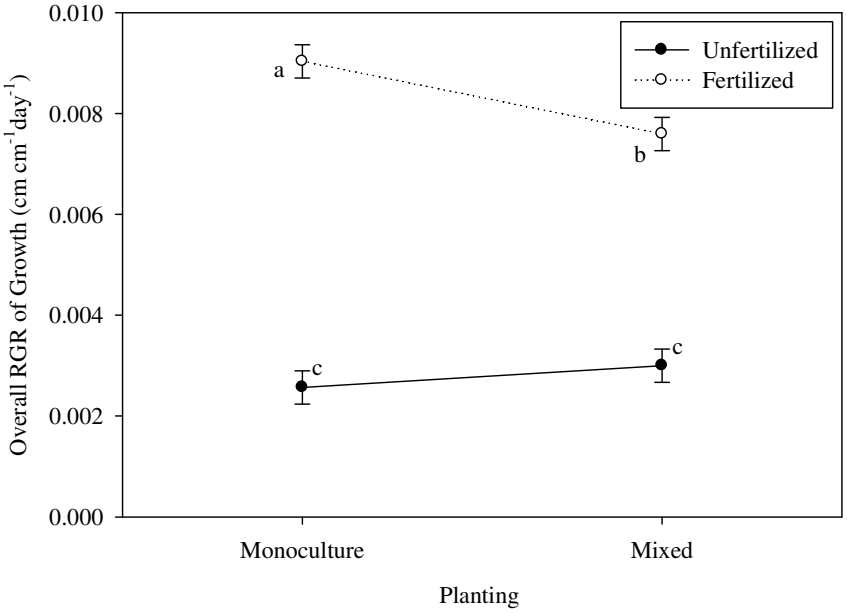



Figure 3.12: *S. cynosuroides* overall relative growth rate calculated with June and December stem height measurements in varying levels of nitrogen additions and competition with *P. australis*. Plotted values are least square means, different lowercase letters denote significant differences in values, and error bars are standard error ($\alpha = 0.05$, $p = 0.0082$).

*Average RGR*

*Phragmites australis'* average RGR was increased with addition of urea fertilizer compared to unfertilized mesocosms ($\alpha = 0.05$, $p < 0.0016$). The growth of *P. australis* was also increased in mixture by an average difference of 0.00206 cm $cm^{-1}$ $day^{-1}$ compared to the growth in monoculture mesocosms ($\alpha = 0.05$, $p < 0.0001$) (Table 3.4, see appendix E-2).

Table 3.4: ANOVA summary for *P. australis*' average RGR, which was calculated from stem height measurements taken in June, July, September, and December 2010 for all treatments.

| Source | ndf, ddf | F | p |
|---|---|---|---|
| Salinity Regime | 3, 30 | 1.48 | 0.2398 |
| Nitrogen | 1, 30 | 173.19 | **<0.0001** |
| Competition | 1, 30 | 12.00 | **0.0016** |
| Salinity Regime x Nitrogen | 3, 30 | 0.27 | 0.8447 |
| Salinity Regime x Competition | 3, 30 | 0.24 | 0.8692 |
| Nitrogen x Competition | 1, 30 | 0.18 | 0.6713 |
| Salinity Regime x Nitrogen x Competition | 3, 30 | 0.35 | 0.7923 |

Bolded values indicate significance at $\alpha = 0.05$.

As seen with the overall RGR, *S. cynosuroides* had higher RGR in the fertilized treatments than unfertilized mesocosms. There was only a significant interaction between fertilization and competition at the alpha = 0.10 level (p = 0.0505) (Figure 3.13).

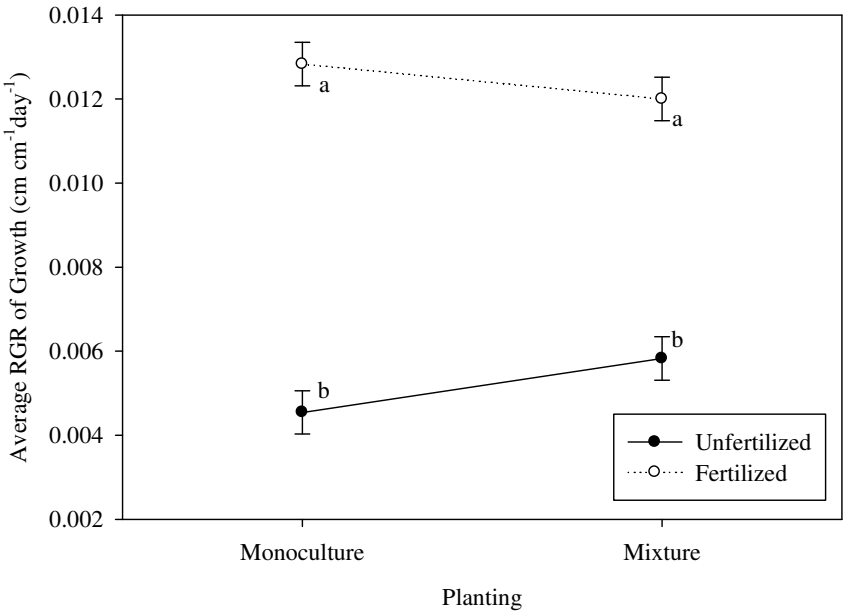



Figure 3.13: Average relative growth rate of *S. cynosuroides* in differing planting treatments and nitrogen fertilization levels. Plotted values are least square means, and different lowercase letters indicate significantly different values. Error bars shown are standard error (α = 0.10, p = 0.0505).

## Biomass

### *Final Aboveground Biomass*

There was a significant interaction of the aboveground biomass growth of *P. australis* between the effects of competition and fertilization (α = 0.05, p = 0.0112) (Figure 3.14, see appendix E-3). The aboveground biomass of *P. australis* was positively affected by the addition of *S. cynosuroides* in fertilized mesocosms by increasing aboveground biomass growth of *P. australis* compared to growth within monoculture mesocosms. In unfertilized mesocosms, there was no significant difference between the levels of competition of aboveground biomass growth of *S. cynosuroides*.

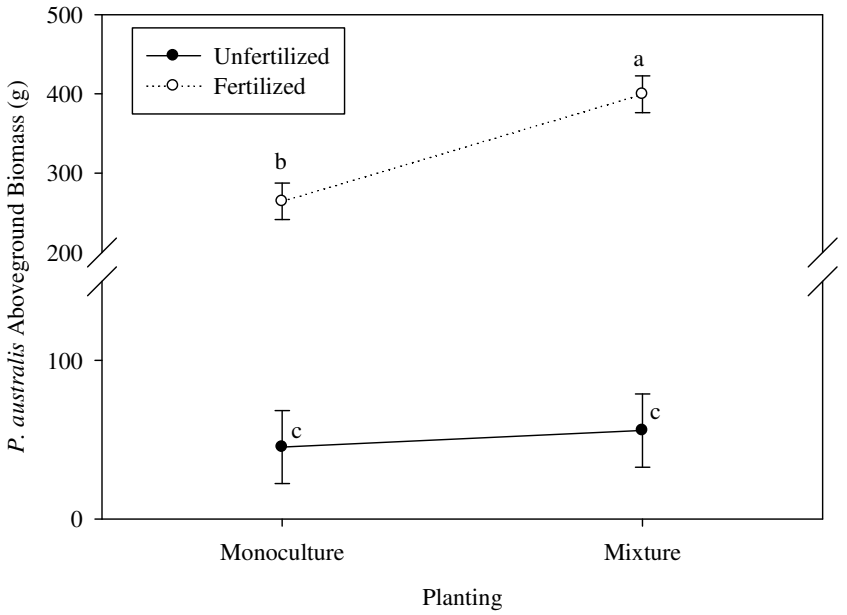



Figure 3.14: Aboveground biomass of *P. australis* in varying levels of nitrogen additions and competition with *S. cynosuroides*. Biomass values of *P. australis* in monoculture were adjusted by dividing by two to compare to mixed biomass values. Plotted values are least square means, different lowercase letters indicate significant differences and error bars are standard error ($\alpha = 0.05$, $p = 0.0112$).

The interaction of salinity regime, competition, and nitrogen was only significant at the alpha equals 0.10 level ($p = 0.0705$) on the aboveground growth of *S. cynosuroides* (Figure 3.15). In fertilized mixed mesocosms with salinity levels of A and B have significantly different values than all of the unfertilized mesocosms, except for the unfertilized, mixed mesocosm at the C salinity level. In salinity levels C and D, monoculture, fertilized mesocosms have significantly more biomass than the unfertilized mesocosms except for the unfertilized, mixed mesocosm at the C salinity level. The monoculture, unfertilized mesocosms at the A salinity level was significantly lower than all of the fertilized mesocosms except for the fertilized, monoculture mesocosms at the C salinity level.

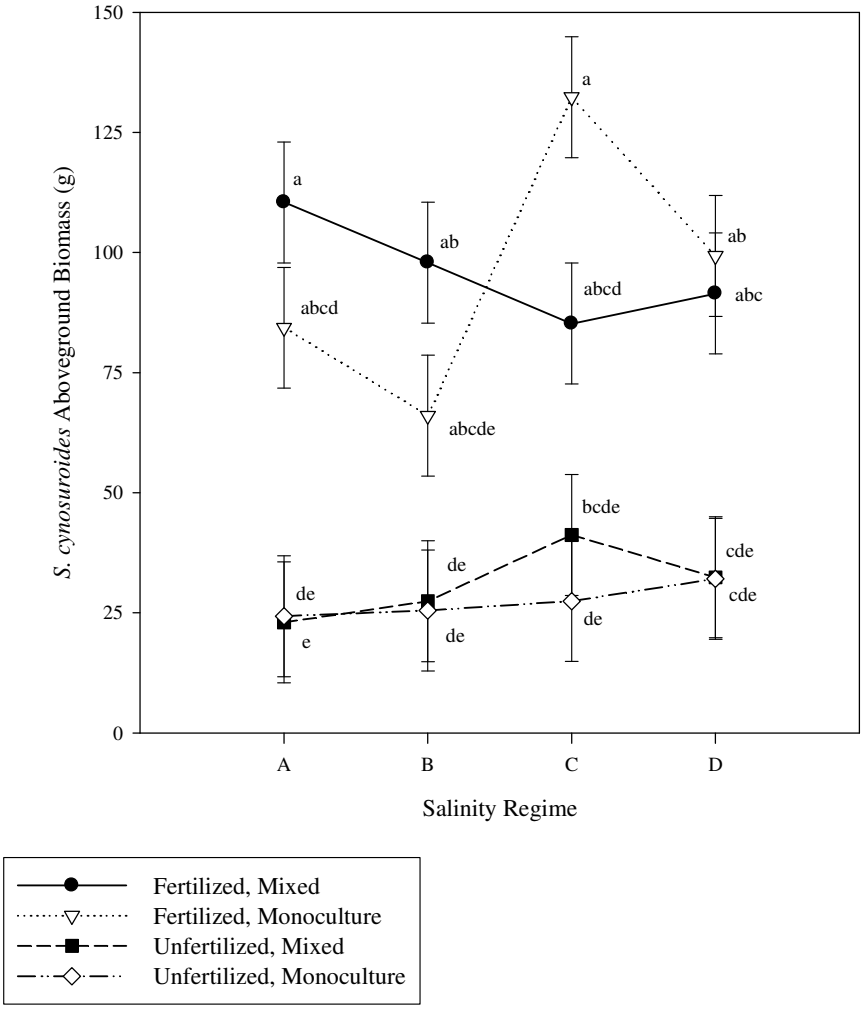



Figure 3.15: Aboveground biomass comparison of *S. cynosuroides* in varying levels of nitrogen and competition in different salinity regimes. Biomass values of *S. cynosuroides* in monoculture were adjusted by dividing by two to compare to mixed biomass values. Salinity levels increase from A to D. Plotted values are least square means, different lowercase letters denote significantly different values, and error bars are standard error ($\alpha = 0.10$ $p = 0.0705$).

*Belowground Biomass*

The addition of nitrogen fertilizer increased belowground biomass in *P. australis* by 2.67 times compared to unfertilized mesocosms ($\alpha = 0.05$, $p < 0.0001$) (see appendix E-4). The amount of belowground biomass of *S. cynosuroides* was significantly affected by the three treatments, salinity regime, fertilization, and competition resulting in a significant three-way interaction ($\alpha = 0.05$, $p < 0.0001$) (Figure 3.16). In the unfertilized

mixed mesocosms, the salinity regime did not affect the belowground biomass growth of *S. cynosuroides*. The fertilized monoculture mesocosms of *S. cynosuroides* had significantly more biomass than the mixture mesocosm especially at the higher salinity regimes, C and D. All of the mixture mesocosms were not significantly affected by the different salinity regimes.

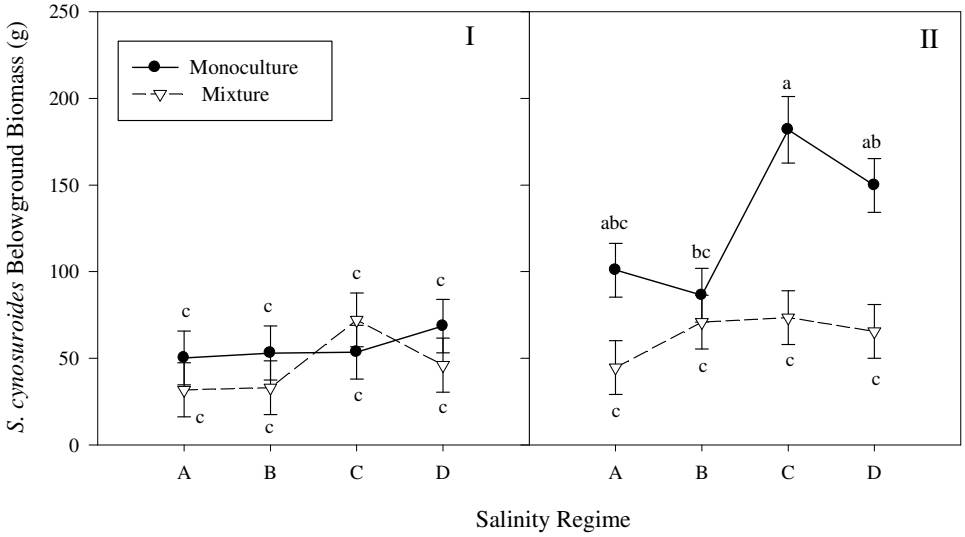



Figure 3.16: Belowground biomass of *S. cynosuroides* in varying levels of nitrogen and competition in different salinity regimes. Biomass values of *S. cynosuroides* in monoculture were adjusted by dividing by two to compare to mixed biomass values. Salinity levels increase from A to D. I. Unfertilized mesocosms of *S. cynosuroides*. II. Fertilized mesocosms of *S. cynosuroides*. Plotted values are least square means, different lowercase letters denote significantly different values, and error bars are standard error ($\alpha = 0.05$, $p < 0.0001$).

The rooting depth patterns significantly differed between *P. australis* and *S. cynosuroides* ($\alpha = 0.05$, $p < 0.0001$) (Figure 3.17). Generally, *P. australis* had a higher belowground biomass than *S. cynosuroides*, especially in the lower depths, 20-40 cm. Both species had the highest biomass in the top ten centimeters of the soil and then decreased with the increase of depth with the exception of *P. australis* at the lowest depth. *S. cynosuroides* severely drops off in biomass below the 20 centimeter depth and had very little biomass in the bottom ten centimeters.

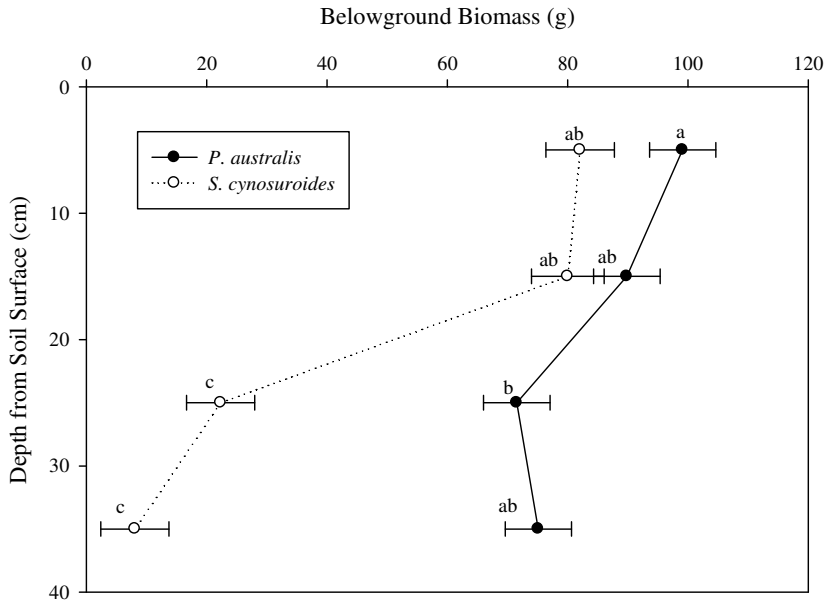



Figure 3.17: Monoculture mesocosms rooting depth patterns comparison by 10-cm depth increments of each species, *P. australis* and *S. cynosuroides*. Plotted values are least square means, different lowercase letters indicate significant differences between values, and error bars are standard error ($\alpha = 0.05$, $p < 0.0001$).

The effects of differing salinity regimes and nitrogen additions on rooting depth patterns were also examined for each species. There was a significant interaction between the rooting depth and the fertilization treatment on the rooting depth pattern of *P. australis* ($\alpha = 0.05$, $p = 0.0221$) (Figure 3.18). There was no difference with depth of the amounts of belowground biomass growth of *P. australis* in unfertilized mesocosms. Alternatively, *P. australis* in the fertilized mesocosms had a general decreasing trend with increasing depth that was significantly different between the surface 10 cm and the bottom 20 cm.

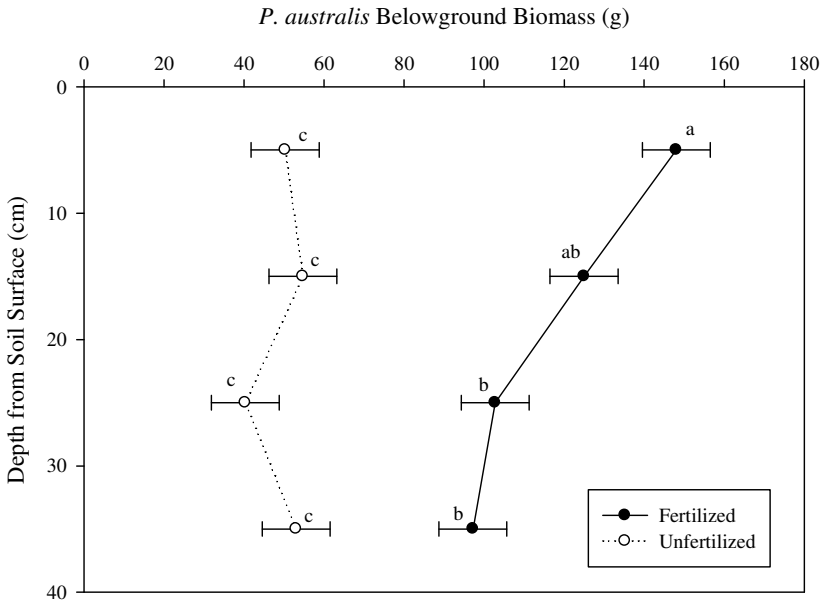



Figure 3.18: Rooting depth pattern comparison of monoculture mesocosms by 10-cm depth increments for *P. australis* in varying levels of fertilization. Plotted values are least square means, different lowercase letters indicate significantly different values and error bars are standard error ($\alpha = 0.05$, $p = 0.0221$).

The interaction of fertilization and rooting depth significantly affected the rooting depth pattern of *S. cynosuroides* ($\alpha = 0.05$, $p < 0.0001$) (Figure 3.19). *Spartina cynosuroides'* rooting depth pattern drastically drops off in the lower 20 cm depth in comparison to the higher 20 cm in both fertilization levels. In the unfertilized mesocosms, the total belowground biomass was generally lower than the fertilized mesocosms. Additionally, in the unfertilized mesocosms the highest biomass was at the 10-20 cm level and the lowest was in the 30-40 cm level. In the fertilized mesocosms, there was a greater drop off from the 10-20 cm level to the 20-30 cm level than in the unfertilized mesocosms.

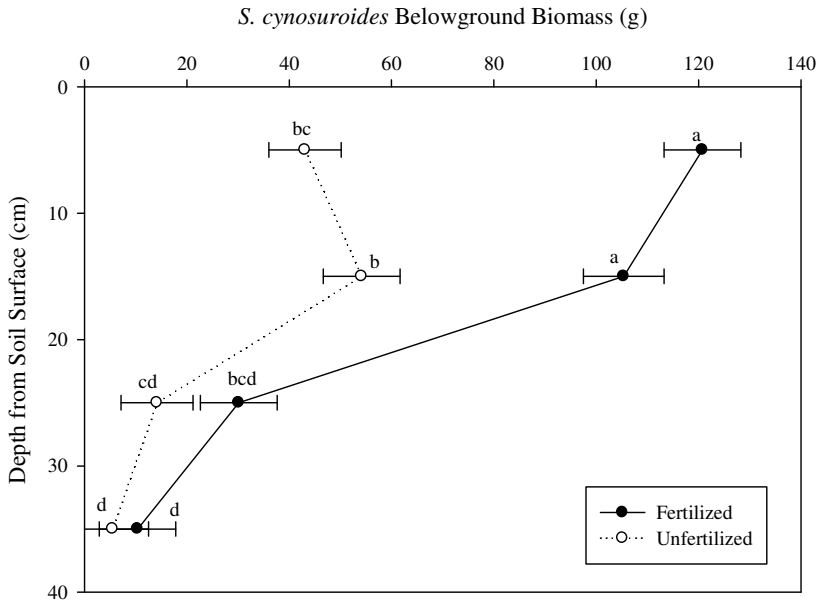



Figure 3.19: Rooting depth pattern comparison of monoculture mesocosms by 10-cm depth increments for *S. cynosuroides* in varying levels of fertilization. Plotted values are least square means, different lowercase letters indicate significantly different values, and error bars are standard error ($\alpha = 0.05$, $p < 0.0001$).

The root to shoot ratio was calculated using the total aboveground and belowground biomass data collected. Both *P. australis* and *S. cynosuroides'* root to shoot ratios were significantly affected by competition ($\alpha = 0.05$, *P. aus.* p=0.0261, *S. cyn.* $p < 0.0001$), and the fertilization treatment ($\alpha = 0.05$, *P. aus.* $p < 0.0001$, *S. cyn.* $p < 0.0001$). However, neither the effects of salinity nor any of the interaction effects between competition, salinity and fertilization were significant. The higher monoculture values in both species indicate that there was more belowground biomass than aboveground biomass, whereas in mixture, *S. cynosuroides* had a decrease in root biomass and a slight change in shoot biomass causing a root to shoot ratio closer to one. *P. australis* in mixture had more shoots than in monoculture creating a lower ratio. In the fertilized treatments, there were more roots than shoots in comparison to the unfertilized treatments in both species. The unfertilized mesocosms had about two times the biomass in the

belowground biomass than the aboveground biomass. In the fertilized mesocosms, both species had a relatively equal number of roots to shoots (Table 3.5).

Table 3.5: Root to shoot ratios of *P. australis* and *S. cynosuroides* in varying levels of nitrogen and competition.

| **Plant Species** | ***Planting*** | | ***Nitrogen Treatment*** | |
|---|---|---|---|---|
| | **Monoculture** | **Mixture** | **Fertilized** | **Unfertilized** |
| *P. australis* | 1.58 ±0.07* | 1.33±0.07* | 0.82±0.07* | 2.09±0.07* |
| *S. cynosuroides* | 1.73 ±0.10* | 1.06±0.09* | 1.02±0.10* | 1.76±0.09* |

Values are arithmetic mean ± SE. * indicates significance at $\alpha = 0.05$ within each species planting and fertilization treatments.

**Chlorophyll Fluorescence**

The leaf fluorescence ($F_v/F_m$) measurements taken for *P. australis* were only significantly affected by the fertilization treatment. $F_v/F_m$ values of *P. australis* were higher in the fertilized mesocosms than the unfertilized mesocosms ($\alpha = 0.05$, $p = 0.0486$). The *S. cynosuroides* $F_v/F_m$ ratios were only significantly affected by the interaction between the salinity regime and fertilization at the alpha level equal to 0.10 ($p = 0.0846$) (Figure 3.20). There were no significant differences seen from the Tukey HSD analysis between the means.

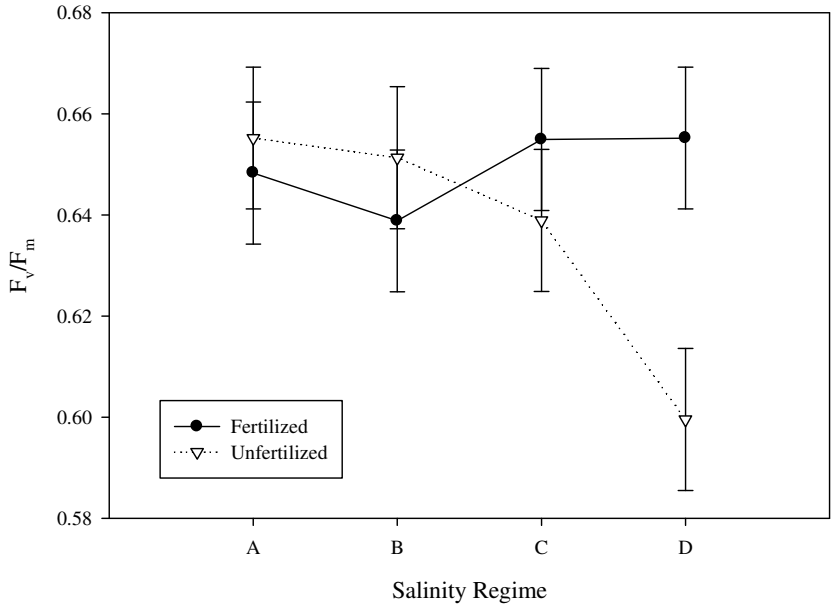



Figure 3.20: $F_v/F_m$ (leaf fluorescence) values of *S. cynosuroides* in varying levels of fertilization and differing salinity regimes. Values are arithmetic means. Error bars are standard error ($\alpha = 0.10$, $p = 0.0846$).

**Replacement Diagrams**

Yields were calculated with total biomass which is comprised of above and belowground biomass as described in Snaydon (1991). In the fertilized mesocosms, the competitive effect is emphasized in both species compared to the unfertilized mesocosms. In all fertilized mesocosms, *P. australis* was facilitated by *S. cynosuroides* (Figure 3.21). In lower salinity levels, A and B, *S. cynosuroides* was unaffected by the competition with *P. australis* in the fertilized treatments. However, in the higher salinity levels, C and D, *S. cynosuroides* was slightly suppressed by the presence and growth of *P. australis.* In the unfertilized mesocosms, both species were generally unaffected by the presence of one another (Figure 3.22). There is a slight indication that the growth of *S. cynosuroides* is facilitating the growth of *P. australis* in the C salinity regime.

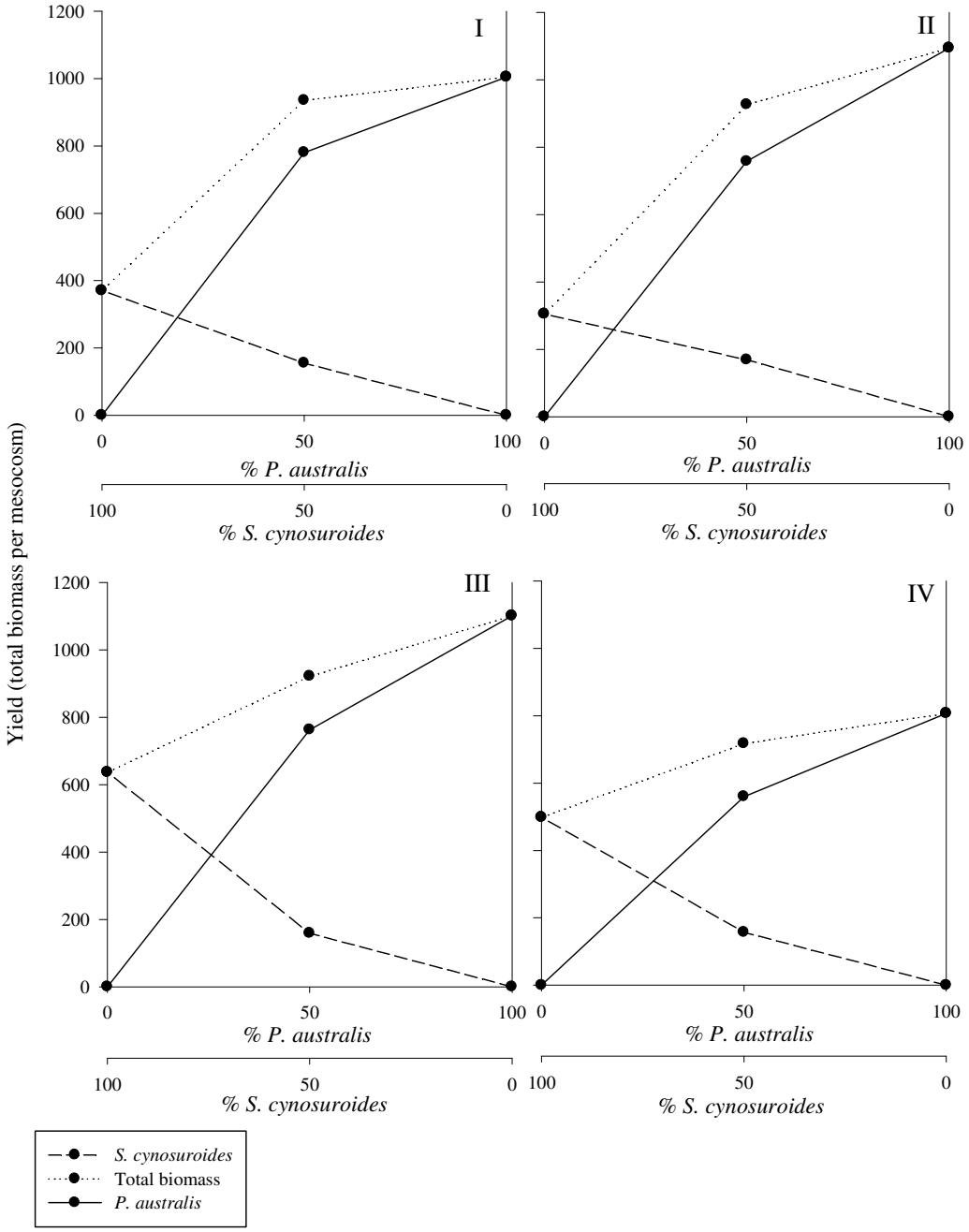



Figure 3.21: Replacement diagrams visualizing the specific yields of each species, *P. australis* and *S. cynosuroides*, in fertilized mesocosms subjected to four different salinity regimes. I. A Salinity Regime II. B Salinity Regime. III. C Salinity Regime. IV. D Salinity Regime. Yield is the sum of the arithmetic mean of the belowground biomass and the arithmetic mean aboveground biomass for each treatment.

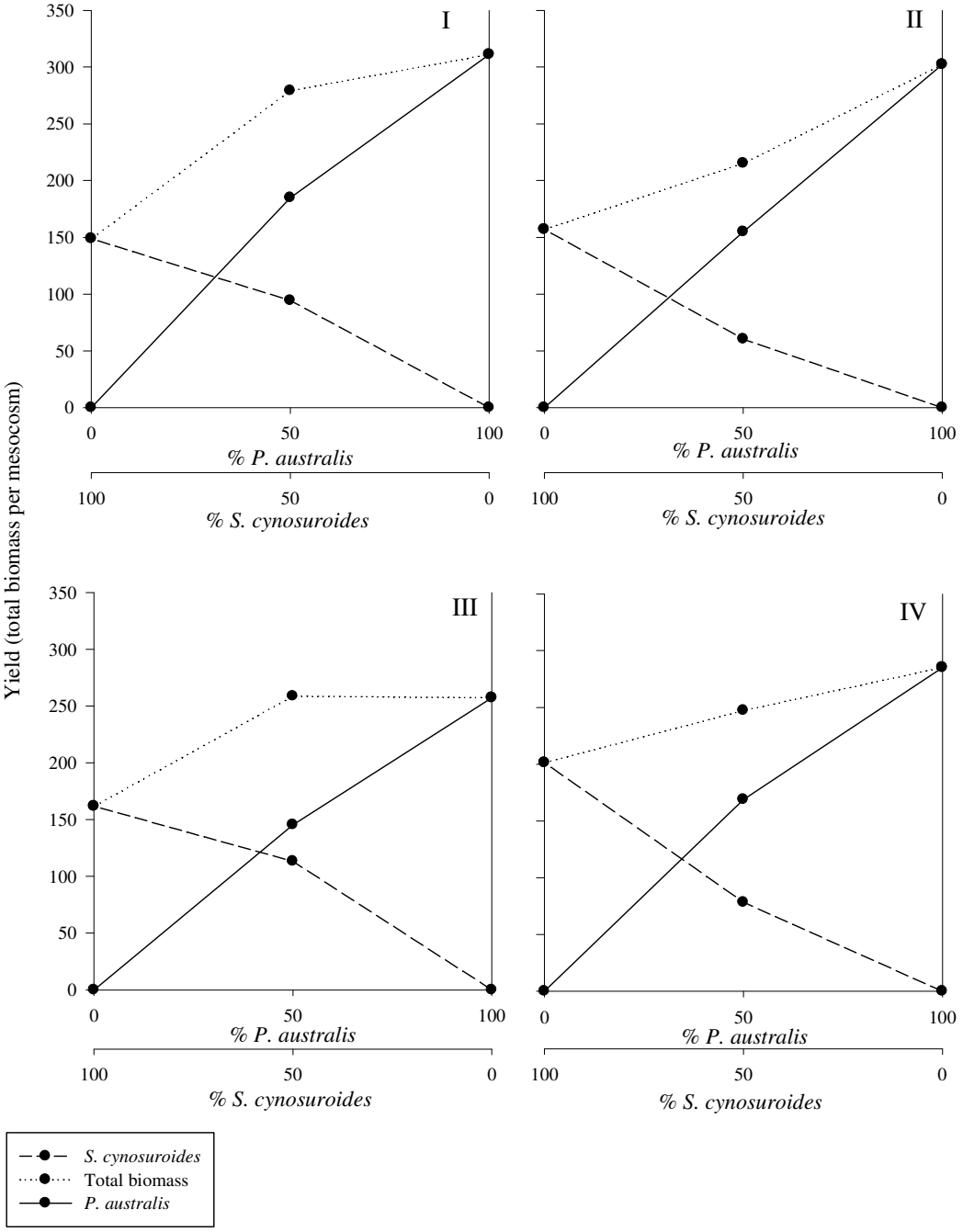



Figure 3.22: Replacement diagrams visualizing the specific yields of each species, *P. australis* and *S. cynosuroides*, in unfertilized mesocosms subjected to four different salinity regimes. I. A Salinity Regime II. B Salinity Regime. III. C Salinity Regime. IV. D Salinity Regime. Yield is the sum of the arithmetic mean of the belowground biomass and the arithmetic mean aboveground biomass for each treatment.

De Wit relative biomass diagrams also allow for the comparison between the levels of competition on a relative scale to each species. As seen with the replacement diagrams, *P. australis'* growth benefitted from the presence of *S. cynosuroides* in fertilization (Figure 3.23). The growth of *P. australis* was generally unaffected by the differences in salinity. However, the growth of *S. cynosuroides* was different at the varying salinity regimes. In the lower salinity regimes, A and B, the growth of *S. cynosuroides* was generally unaffected by the presence of *P. australis.* However, in the higher salinity regimes, C and D, the growth of *S. cynosuroides* was negatively affected by the competition of *P. australis* in mixture. In the unfertilized mesocosms, *P. australis* was not affected by the levels of competition or differing salinity regimes; the relative values are generally consistent in all unfertilized mesocosms (Figure 3.24). The *S. cynosuroides* growth differed based on the different salinity regimes in the unfertilized mesocosms. In the A and C salinity regimes, *S. cynosuroides* appeared to slightly benefit from *P. australis* in mixture. However, in the other salinity regimes, B and D, *S. cynosuroides'* growth was negatively affected by the growth of *P. australis* in mixture by only slight amounts.

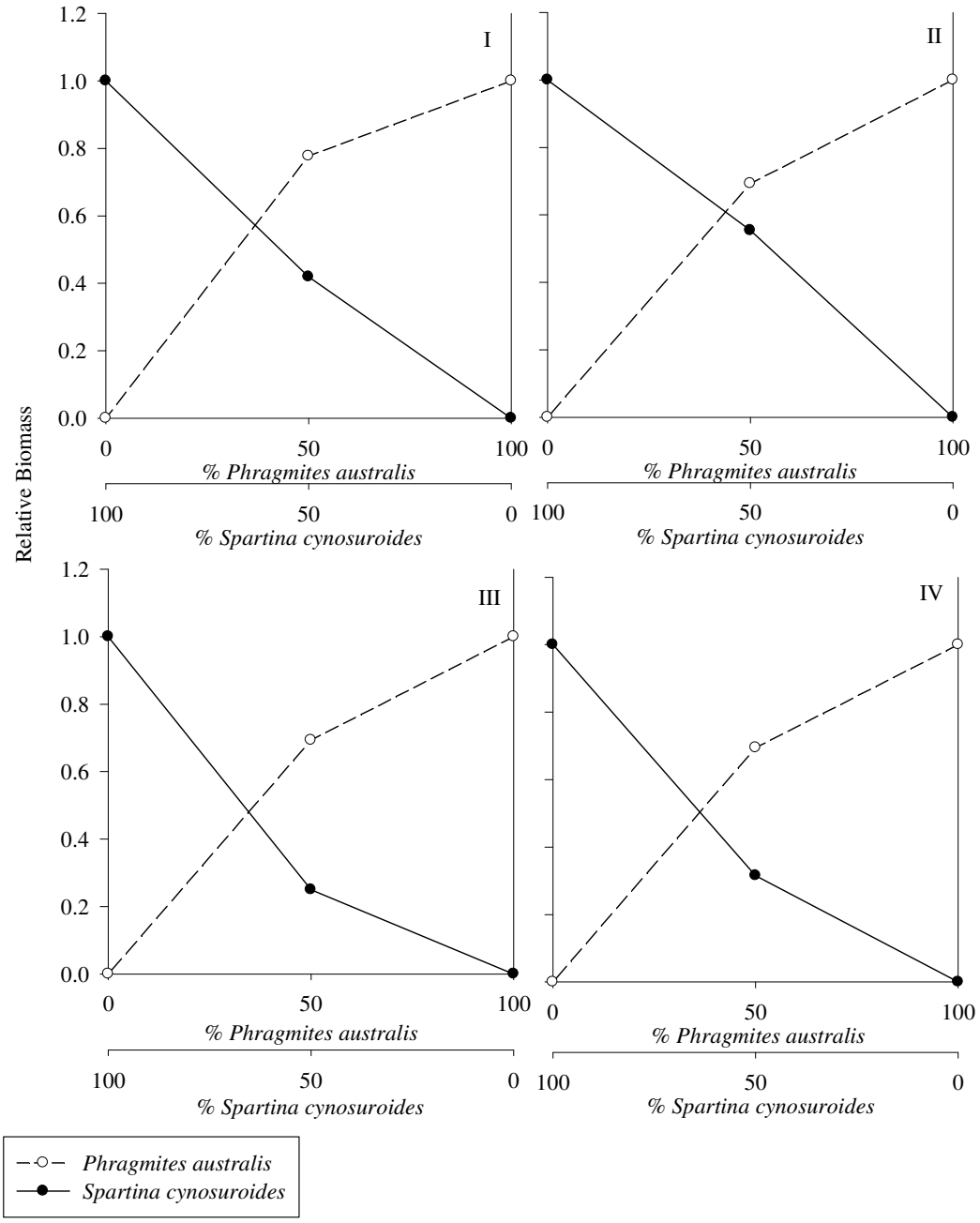



Figure 3.23: De Wit diagrams visualizing the relative yields of each species, *P. australis* and *S. cynosuroides*, in fertilized mesocosms subjected to four different salinity regimes. I. A Salinity Regime II. B Salinity Regime. III. C Salinity Regime. IV. D Salinity Regime. Relative biomass is the sum of the arithmetic mean of the belowground biomass and the arithmetic mean aboveground biomass for each treatment over the relative yield in monoculture.

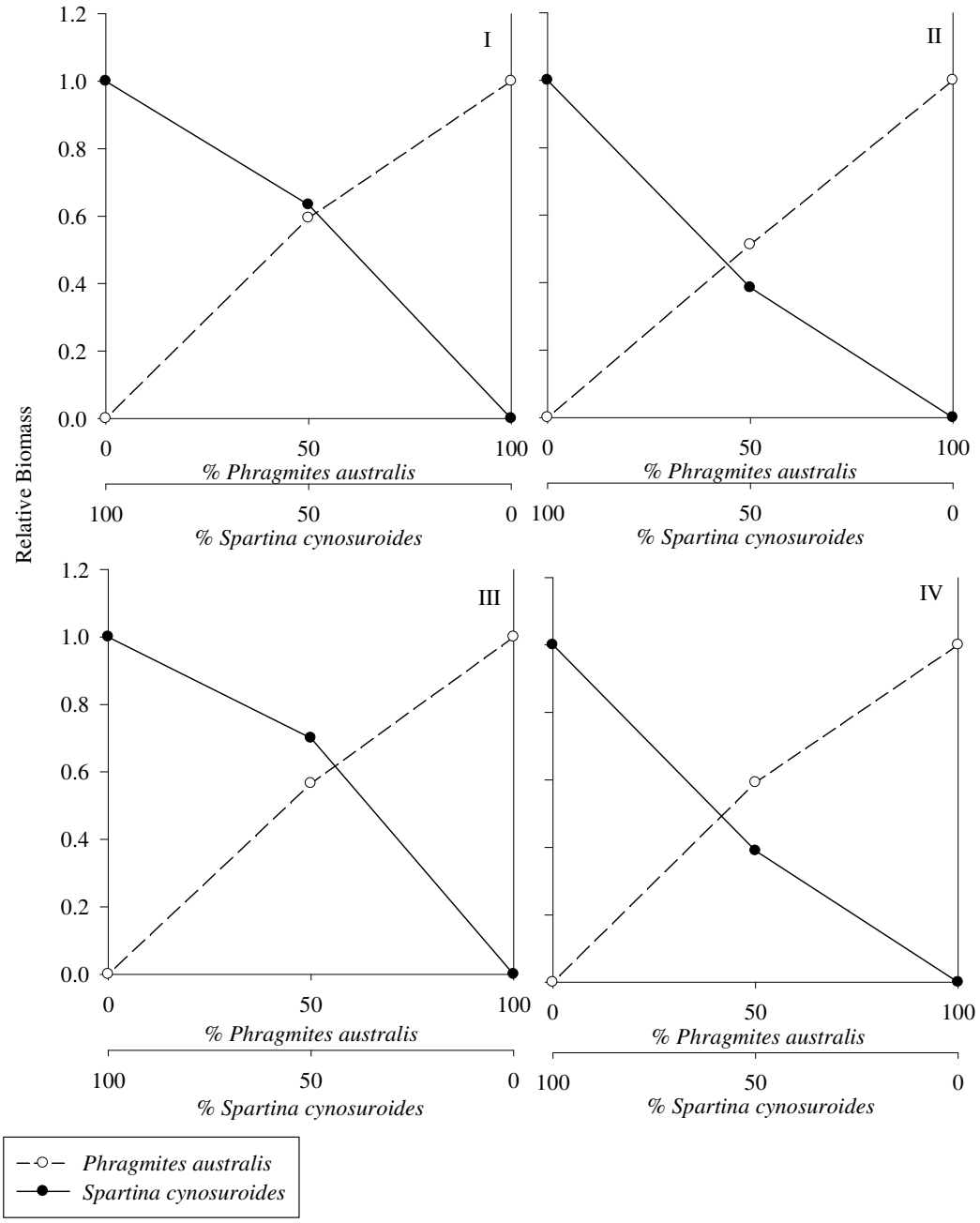



Figure 3.24: De Wit diagrams visualizing the relative yields of each species, *P. australis* and *S. cynosuroides*, in unfertilized mesocosms subjected to four different salinity regimes. I. A Salinity Regime II. B Salinity Regime. III. C Salinity Regime. IV. D Salinity Regime. Relative biomass is the sum of the arithmetic mean of the belowground biomass and the arithmetic mean aboveground biomass for each treatment over the relative yield in monoculture.

**General Observations**

The growth of *S. cynosuroides* differed from *P. australis* in culm distribution. *Phragmites australis'* culms spread out through the entire mesocosms within and around the *S. cynosuroides'* culms. However, the *S. cynosuroides'* culms kept dense patches in the same location where the clonal ramets were initially planted with little or no expansion. This pattern was also seen in a competition study done by Saltonstall and Stevenson (2007) between native and non-native *P. australis*, where the native *P. australis* grew in dense patches similar to the *S. cynosuroides*. Additionally, mesocosms with *P. australis* had very little microtopography, whereas mesocosms *S. cynosuroides* often had deeper holes forming where culms were not present.

**Nondestructive Measurements**

*Early RGR*

To examine the components of the overall and average RGR, early RGR was examined for each species. For *P. australis* there was only a significant effect of competition in the June to September RGR ($\alpha = 0.05$, $p = 0.0349$) where mixture had higher RGR values (estimated value = 0.02432±0.000630 cm $cm^{-1}day^{-1}$) than monoculture (estimated value = 0.02235±0.000630 cm $cm^{-1}day^{-1}$) (Table 3.6). There was a higher RGR in mixture than in monoculture during this time period. Fertilization had a significant effect on the RGR of *P. australis* in all three time periods where there was higher RGR in fertilized mesocosms than unfertilized mesocosms (Table 3.7) ($\alpha = 0.05$, $p < 0.0001$). There were no significant interactions of the treatments on the early RGR of *P. australis.*

Table 3.6: ANOVA summary for *P. australis* for early RGR measurements from stem height measurements taken in June, July, and September 2010 for all treatments.

| Source | ndf, ddf | F | p |
|---|---|---|---|
| ***June to July RGR*** | | | |
| Salinity Regime | 3, 30 | 1.91 | 0.1499 |
| Nitrogen | 1, 30 | 37.58 | <0.0001 * |
| Competition | 1, 30 | 1.65 | 0.2086 |
| Salinity Regime x Nitrogen | 3, 30 | 0.35 | 0.7873 |
| Salinity Regime x Competition | 3, 30 | 0.39 | 0.7640 |
| Nitrogen x Competition | 1, 30 | 0.34 | 0.5667 |
| Salinity Regime x Nitrogen x Competition | 3, 30 | 0.58 | 0.6349 |
| ***July to September RGR*** | | | |
| Salinity Regime | 3, 30 | 0.21 | 0.8868 |
| Nitrogen | 1, 30 | 53.14 | <0.0001 * |
| Competition | 1, 30 | 2.62 | 0.1158 |
| Salinity Regime x Nitrogen | 3, 30 | 0.47 | 0.7082 |
| Salinity Regime x Competition | 3, 30 | 0.22 | 0.8824 |
| Nitrogen x Competition | 1, 30 | 0.20 | 0.6594 |
| Salinity Regime x Nitrogen x Competition | 3, 30 | 0.54 | 0.6568 |
| ***June to September RGR*** | | | |
| Salinity Regime | 3, 30 | 0.41 | 0.7479 |
| Nitrogen | 1, 30 | 103.07 | <0.0001 * |
| Competition | 1, 30 | 4.88 | 0.0349 * |
| Salinity Regime x Nitrogen | 3, 30 | 0.23 | 0.8753 |
| Salinity Regime x Competition | 3, 30 | 0.19 | 0.9013 |
| Nitrogen x Competition | 1, 30 | 0.00 | 0.9596 |
| Salinity Regime x Nitrogen x Competition | 3, 30 | 0.28 | 0.8370 |

* indicates significance at $\alpha = 0.05$, ** indicates significance at $\alpha = 0.10$.

Table 3.7: The effects of nitrogen fertilization on the relative growth rate (RGR) of *P. australis* in the early months based on the stem height measurements taken in June, July, and September 2010.

| Nitrogen Level | June to July RGR (cm $cm^{-1}day^{-1}$) | July to September RGR (cm $cm^{-1}day^{-1}$) | June to September RGR (cm $cm^{-1}day^{-1}$) |
|---|---|---|---|
| **Fertilized** | 0.04767±0.001279 | 0.01961±0.000796 | 0.02786±0.000630 |
| **Unfertilized** | 0.03659±0.001279 | 0.01140±0.000796 | 0.01881±0.000630 |

For *S. cynosuroides*, there was only a significant effect of competition in the June to July RGR at the alpha equals to 0.10 level (p = 0.0564) (Table 3.8). In this time period, *S. cynosuroides* had a higher RGR in mixture (estimated value = 0.02077±0.001232 cm $cm^{-1}day^{-1}$) than in monoculture (estimated value = 0.01732±0.001232 cm $cm^{-1}day^{-1}$). Additionally from June to July, the fertilization treatment had a significant effect on the

RGR, where fertilized mesocosms had a RGR of 0.02487±0.001232 cm $cm^{-1}day^{-1}$ and unfertilized mesocosms' RGR was 0.01322±0.001232 cm $cm^{-1}day^{-1}$.

Table 3.8: ANOVA summary for *S. cynosuroides* for early RGR measurements from stem height measurements taken in June, July, and September 2010 for all treatments.

| Source | ndf, ddf | F | p |
|---|---|---|---|
| ***June to July RGR*** | | | |
| Salinity Regime | 3, 30 | 0.56 | 0.6467 |
| Nitrogen | 1, 30 | 44.64 | <0.0001 * |
| Competition | 1, 30 | 3.94 | 0.0564 ** |
| Salinity Regime x Nitrogen | 3, 30 | 1.84 | 0.1612 |
| Salinity Regime x Competition | 3, 30 | 1.07 | 0.3748 |
| Nitrogen x Competition | 1, 30 | 0.31 | 0.5804 |
| Salinity Regime x Nitrogen x Competition | 3, 30 | 0.59 | 0.6268 |
| ***July to September RGR*** | | | |
| Salinity Regime | 3, 30 | 0.45 | 0.7180 |
| Nitrogen | 1, 30 | 48.06 | <0.0001 * |
| Competition | 1, 30 | 4.75 | 0.0372 * |
| Salinity Regime x Nitrogen | 3, 30 | 1.89 | 0.1524 |
| Salinity Regime x Competition | 3, 30 | 0.86 | 0.4750 |
| Nitrogen x Competition | 1, 30 | 3.03 | 0.0921 ** |
| Salinity Regime x Nitrogen x Competition | 3, 30 | 0.88 | 0.4610 |
| ***June to September RGR*** | | | |
| Salinity Regime | 3, 30 | 0.60 | 0.6197 |
| Nitrogen | 1, 30 | 144.60 | <0.0001 * |
| Competition | 1, 30 | 1.09 | 0.3047 |
| Salinity Regime x Nitrogen | 3, 30 | 0.59 | 0.6272 |
| Salinity Regime x Competition | 3, 30 | 1.24 | 0.3113 |
| Nitrogen x Competition | 1, 30 | 5.15 | 0.0306 * |
| Salinity Regime x Nitrogen x Competition | 3, 30 | 1.11 | 0.3608 |

* indicates significance at $\alpha = 0.05$, ** indicates significance at $\alpha = 0.10$.

In the July to September RGR measurements, *S. cynosuroides* had only a significant effect of the interaction of fertilization and competition at the alpha equals to 0.10 level ($p = 0.0921$). However, the main effect of competition on *S. cynosuroides* RGR was significant at the alpha equals 0.05 level during the time period of July to September.

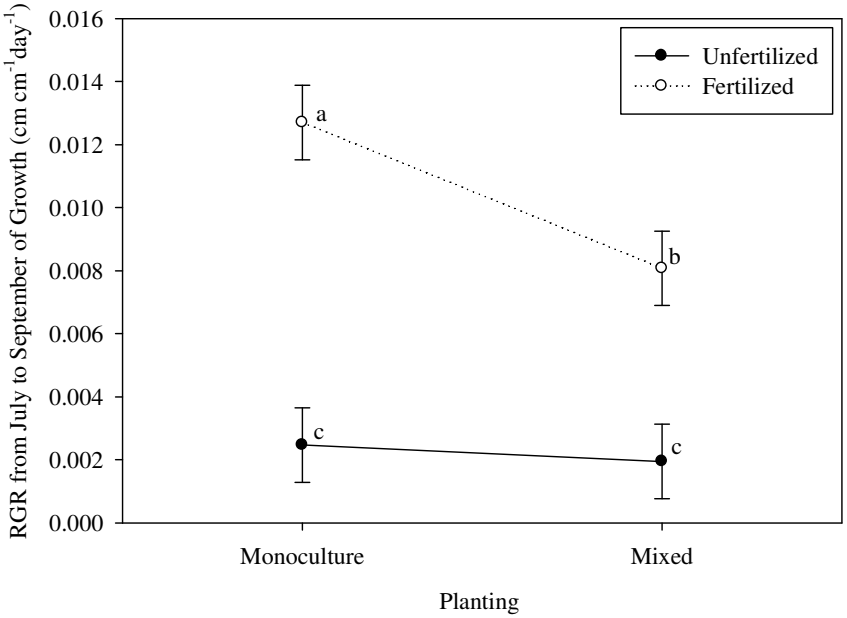



Figure 3.25: *S. cynosuroides'* relative growth rate calculated between July and September stem height measurements in varying levels of nitrogen additions and competition with *P. australis*. Plotted values are least square means, different lowercase letters denote significant differences in values, and error bars are standard error ($\alpha = 0.10$, $p = 0.0921$).

During the overall early time period of June to September, the RGR was significantly affected by the interaction between fertilization and competition ($\alpha = 0.05$, $p = 0.0306$) (Figure 3.26). The main effect of fertilization had a significant effect on the RGR of *S. cynosuroides* in all three time periods ($\alpha = 0.05$, $p < 0.0001$).

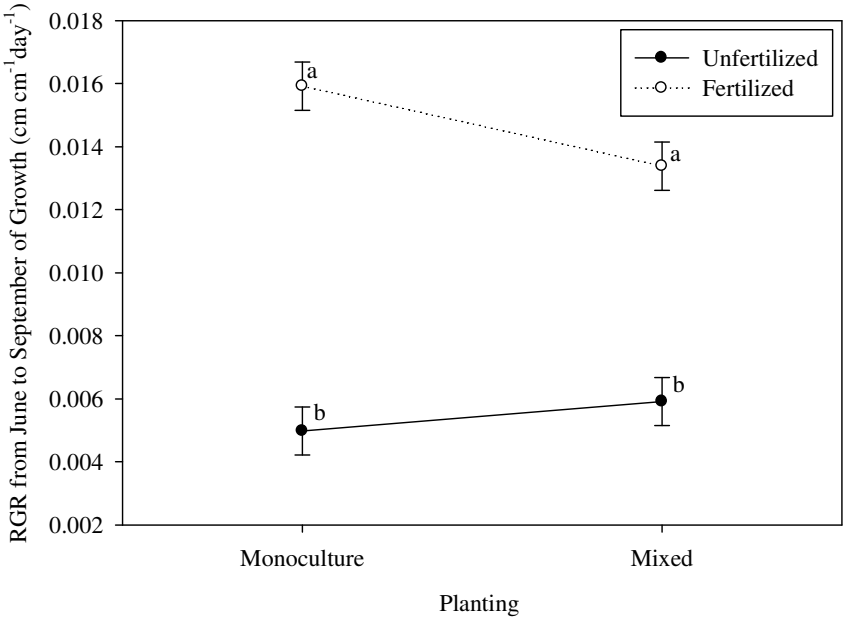



Figure 3.26: *S. cynosuroides'* relative growth rate calculated between June and September stem height measurements in varying levels of nitrogen additions and competition with *P. australis*. Plotted values are least square means, different lowercase letters denote significant differences in values, and error bars are standard error ($\alpha = 0.05$, $p = 0.0306$).

*Extrapolated September Aboveground Biomass*

To ensure that the results found in December biomass calculations were not affected by pot bounding, an extrapolation of the relationship between total stem height and total aboveground biomass from December was used to estimate the biomass in September based on measured stem height (Figure 3.27). The allometric relationship found for both species was significant at the alpha equals 0.05 level ($p < 0.0001$).

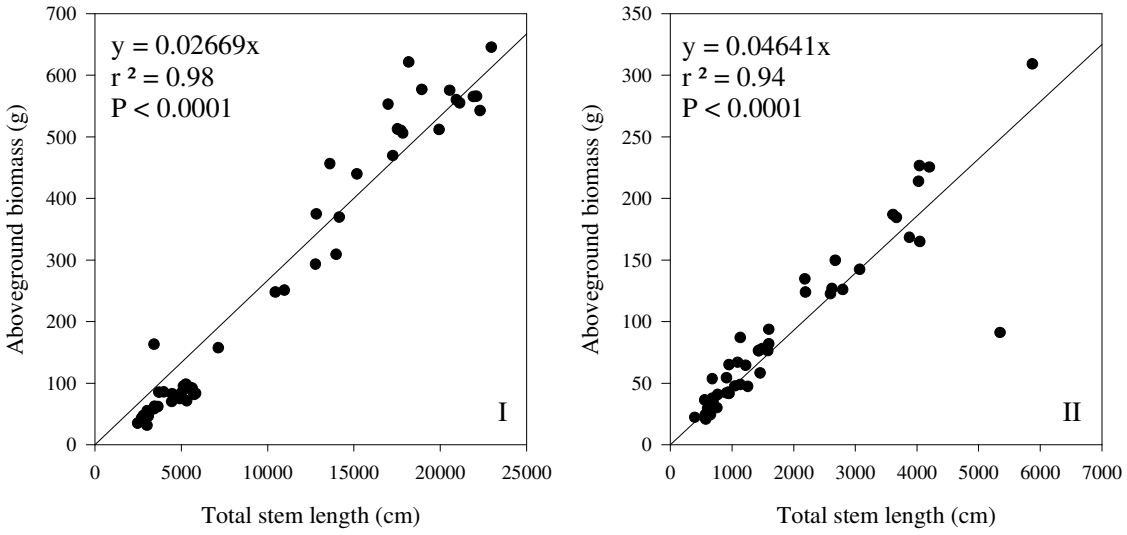



Figure 3.27: Regression analysis to establish an allometric relationship between stem height and aboveground biomass using the data collected in December 2010. Calculations were conducted separately for both species. I. *Phragmites australis* II. *Spartina cynosuroides*. Plotted values are actual measured values.

Estimated aboveground biomass of *P. australis* was significantly affected by the nitrogen treatment, which resulted in over twice the estimated aboveground biomass in fertilized mesocosms than unfertilized mesocosms ($\alpha = 0.05$, $p < 0.0001$). The effect of competition was only significantly affected at the alpha equals 0.10 level, where *P. australis* in monoculture (estimated value = 86.72±6.19 g) had less biomass than it in mixture (estimated value = 104.59±6.19 g). The only significant effect on the aboveground biomass growth of *S. cynosuroides* was the nitrogen treatment, where there was twice the aboveground biomass growth in fertilized treatments than in unfertilized treatments ($\alpha = 0.05$, $p < 0.0001$).

## DISCUSSION

In all treatments including salinity, fertilization, and competition, *P. australis* developed more relative and absolute biomass than *S. cynosuroides,* indicating that *P. australis* is a more productive species in terms of biomass than *S. cynosuroides* in all of

our treatments. This could have positive implications for wetland persistence because of the high primary productivity, seen in *P. australis* in this and other studies (Findlay et al. 2002, Saltonstall and Stevenson 2007), could assist in the reversal of slowed accretion rates. The rooting depth patterns differed between each species by the amount of biomass by depth; *P. australis* belowground biomass had more biomass in the lower depths than *S. cynosuroides*. Coastal wetland environments could benefit from the deeper rooting patterns of *P. australis* because of the soil retention potential and erosion prevention.

Based on the extrapolated results for the September aboveground biomass, the results were similar to the ones found in the December measurements. This could indicate that there was no effect of pot bounding on the treatments in the last four months of the experiment since there were similar results in September and December.

**Salinity Pulses and Competition**

The differing salinity regimes did not have any significant effects on the growth dynamics of *P. australis* with respect to biomass measurements, RGR, or leaf fluorescence. This could be a result from the increased soil aeration from high belowground biomass growth which then creates lower sulfide concentration thus reducing toxicity (Chambers et al. 1998, Burdick et al. 2001) or the fluctuations in the levels of salinity. The only factor that was significantly affected by the salinity regimes was the belowground growth of *S. cynosuroides*. Belowground biomass was significantly higher in the C and D salinity regimes of fertilized monoculture mesocosms compared to A and B salinity regimes of fertilized monocultures and all unfertilized and mixed mesocosms. The growth of *P. australis* had a significant effect on the

belowground growth of *S. cynosuroides* in the higher salinities by suppressing *S. cynosuroides* growth. One reason for the lack of competition effect of *S. cynosuroides* in lower salinities could be due to the low sulfate concentration. Stribling (1997) and Bradley and Dunn (1989) proposed that sulfate might be required for higher *S. cynosuroides* growth for osmotic adjustments. The aboveground growth of *S. cynosuroides* had no significant differences between means but did have a significant three-way interaction effect of salinity, fertilization, and competition. There was no significant difference between the potential quantum efficiency levels in the different salinity regimes even though there was a significant interaction between salinity regimes and fertilization. This lack of significance could be attributed to chlorophyll fluorescence's variable values and insensitivity to wetland species which have been seen in other studies (Pearcy and Ustin 1984) or that these values represent a relative stress and not a specific value.

**Fertilization and Competition**

Both species had more biomass when fertilized with nitrogen, which has been seen in a similar fertilization study by Rickey & Anderson (2004) with *P. australis.* *Phragmites australis* also had significantly more growth than *S. cynosuroides*. The higher levels of fertilization affected the rooting depth patterns of both species. In the mesocosms without nitrogen, the highest biomass was seen at lower depths of their bulk of belowground biomass. So for *S. cynosuroides*, this was in the 10-20cm depth range and for *P. australis* the higher biomass was in the 30-40cm depth range. This growth could be attributed to the expansion needed to find adequate nutrient sources. Consequently, the mesocosms treated with nitrogen had the highest belowground

biomass in the top 10 cm. This allows for *P. australis,* and probably *S. cynosuroides*, to shift its expansion efforts toward aboveground growth and not in nutrient finding belowground growth. This was seen in a similar study done on *P. australis* by Minchinton and Bertness (2003). The lowered belowground biomass could be a leading factor in marsh collapse due to the decrease in accretion and an increase in erosion and flooding as suggested in other studies (Mendelssohn et al. 1981, Hartig et al. 2002, Darby and Turner 2008).

The effects of increased biomass of *P. australis* were seen in the belowground and aboveground growth including biomass and RGR of *S. cynosuroides* in mixed mesocosms. Typically the rapid growth of *P. australis* creates a highly competitive environment for native species to inhabit because of shading and space constraints, as demonstrated in other studies (Levine et al. 1998, Saltonstall and Stevenson 2007). Due to the effect of competition, the RGR of fertilized *S. cynosuroides* were lower in mixture than in monoculture. The effect of shading from *P. australis* could have affected the growth of aboveground biomass of *S. cynosuroides* in mixture. Parrondo et al. (1978) showed that *S. cynosuroides* had higher amounts of belowground and aboveground biomass in lower concentrations of NaCl (0 and 4 g/L) compared to the higher concentrations used (8,16, and 32 g/L). So, the reason there could be a lack of competition effect of *P. australis* on *S. cynosuroides* in lower salinities is because there might not be an enough sulfate concentration for higher *S. cynosuroides* growth as mentioned previously (Bradley and Dunn 1989, Stribling 1997). In the unfertilized mesocosms, the effects of competition or salinity may have been minimized because of

the lack of fertilizer. When examining the RGR, it is clear that *P. australis* is facilitated by *S. cynosuroides* especially in high nitrogen environments.

For *P. australis* growth including aboveground growth, RGR values, and root to shoots ratios, there was greater overall biomass in the mixture mesocosms than in the monoculture mesocosms. As indicated by the de Wit diagrams, the facilitation exhibited could be from a lack of competition on *P. australis.* The intra-species competition could be much higher between *P. australis* than the inter-species competition with *P. australis* and *S. cynosuroides* which could create an advantage for *P. australis* in mixture than in monoculture. This facilitation also could be from a productivity feedback loop as suggested by Howes et al. (1986), in which the increased productivity from both species increased the evapotranspiration within the mesocosms creating lower water levels and increased aeration in the top levels of the soil as hypothesized in similar studies (Howes et al. 1986, Chambers et al. 2003). This effect would only have occurred when the mesocosms were not flooded. The increased oxygen levels within the soil may have helped *P. australis*' ability to take up nitrogen and combat the effects of sulfide toxicity thus creating more growth and continuing the productivity feedback loop as other studies have suggested (Linthurst and Seneca 1981, Howes et al. 1986, Chambers 1997, Burdick et al. 2001). In addition, *S. cynosuroides* could have slightly decreased the salinity within the mixed mesocosms by taking up salt and excreting the excess salts through its leaves, which is shown with similar species in other studies (Ewing et al. 1995). The early RGR values show that the effects of competition were not seen in the first month, but it was seen within four months of growth. The lack of effect within the first month could be

attributed to the lack of interaction during that time. However, by the fourth month the species were interacting and the effects of the competition could be seen.

**Root to Shoot Ratio and Competition**

The root to shoot ratio is important variable in the characterization of marsh collapse from increased nutrients. The ratio can quantify the balance of belowground biomass production to aboveground production. A dramatically uneven ratio could indicate the shift from belowground biomass production to aboveground biomass production which could be caused by the increase of nutrient pollution as theorized in other studies (Valiela et al. 1976, Minchinton and Bertness 2003). In mixture *S. cynosuroides* had fewer roots than shoots creating a lower root to shoot ratio, which can probably be attributed to crowding from *P. australis'* belowground biomass growth, which has been described in other studies (Levine et al. 1998, Burdick and Konisky 2003). *Phragmites australis'* lower root to shoot ratio in mixture can be attributed to the increase in aboveground biomass compared to the growth in monoculture, which could indicate a competitive response for increased shading of competitors. There was no effect on the belowground growth of *P. australis* by the presence of *S. cynosuroides.* Additionally, *S. cynosuroides* could have created more favorable growing conditions by the improved nutrient and oxygen availability from the increase of soil aeration at the soil surface. In fertilized mesocosms, there was an equal root to shoot ratio for *S. cynosuroides* and much higher shoot mass in *P. australis*. The high shoot mass in *P. australis* has been seen in other nutrient enrichment studies including Rickey and Anderson (2004) and Saltonstall and Stevenson (2007). The high aboveground growth and low belowground growth illustrates the effects of decreased soil retention in

increased nutrient environments thus leading to unstable conditions that favor erosion as was predicted in Jamaica Bay, NY (Hartig et al. 2002) and further described in Darby and Turner's eutrophication study (2008). This proves Valiela et al. (1976) and Turner et al. (2004) theory of marsh collapse due to the increase of nutrients creating a shift from belowground biomass production to aboveground biomass production. Whereas in the low nutrient environment of the unfertilized mesocosms, there was more belowground biomass than aboveground biomass, but still only half as much belowground biomass as compared to the fertilized mesocosms indicating that some fertilization is required to ensure stabilization.

**Management Potential**

The deep rooting characteristics and adaptability of *P. australis* might prove to be beneficial when the accretion rates are not keeping pace with the increase in sea-level rise and nutrient levels. The ability for *P. australis*, by modifying its surrounding environment, to create conducive growing environments could allow for more sediment retention in wetlands as theorized by Burdick et al. (2001). In addition, the presence of this native species, *S. cynosuroides*, actually facilitated the spread and success of *P. australis*. By facilitating *P. australis* growth, native species could help establish growth and accretion by *P. australis* in submerging wetlands. As a result, *P. australis* could be beneficial in sustaining coastal marsh environments against accelerated sea-level rise and instability from increased nutrients. More research needs to be done to determine how *P. australis* benefits from the presence of *S. cynosuroides* and if *P. australis* could help sustain wetland environments in predicted conditions created by sea-level rise.

# CHAPTER 4

## *Conclusions*

Through the examination of the conditions in the field and experimental environments within the greenhouse, I have explored biomass allocation dynamics of different species in various environmental conditions. From the observational study, I was able to assess the relationship between species diversity and biomass allocation along a salinity gradient. From this information, I was able to observe similar trends that were published in other diversity studies. These studies have found that in low stress inducing environments, competition will dictate the species composition. However, in higher salinity and flooding environments, species diversity is dependent on the species that can survive these unfavorable conditions (Pennings and Callaway 1992, Gough et al. 1994, Crain et al. 2004, Pennings et al. 2005, Sharpe and Baldwin 2009, Engels and Jensen 2010). Additionally I observed that the belowground biomass was higher in the high diversity sites, which could indicate high competition, competitive exclusion, or complementary use of space and nutrients in a facilitative relationship (Bertness 1991, Bertness and Shumway 1993, Bertness and Callaway 1994, Bertness and Hacker 1994, Hooper 1998). I was able to accept my hypothesis that the oligohaline sites of 3 and 4 had the highest number of species per plot. Site 4 did have the highest overall belowground biomass as hypothesized, but site 3 was the second lowest overall belowground biomass and the lowest biomass in the top 10 cm of all of the sites. Through the data collected, I was able to observe the distribution of the biomass allocation based on salinity, which could be beneficial in understanding the reactions of this estuarine environment to sea-level rise and nutrient influxes.

The greenhouse competition experiment allowed me to focus on two particular species and their reactions to predicted conditions of sea-level rise and nutrient-pollution. One of the main purposes of the study was to focus on the competitive interactions between the native species, *S. cynosuroides*, and the non-native species, *P. australis* in these predicted conditions. I hypothesized that the *P. australis* would be more competitive and dominate over the growth of *S. cynosuroides* in the lower salinity levels. However, the results showed that there was not a significant difference between the growth of *S. cynosuroides* in mixture and in monoculture regardless of nutrient levels. At the higher salinities with fertilization, *S. cynosuroides* was negatively affected by the presence of *P. australis,* which was the opposite of my predictions. I predicted that the *S. cynosuroides* would have an advantage over *P. australis* in the higher salinity levels. In all cases, especially in fertilized mesocosms, *P. australis* had more biomass then *S. cynosuroides,* which was hypothesized in the fertilized mesocosms. Based on the lower biomass measurements, reduced relative growth rates, and decreased leaf fluorescence, both species were stressed by the lack of fertilizer in the unfertilized mesocosms. Derived from the data collected, there could be some beneficial qualities of the non-native *P. australis* for organic matter additions in the soil. This species could counteract the effects of marsh collapse in terms of increasing organic matter in the system and soil retention due to its deep rooting systems.

**Recommended Future Studies**

These two studies open up a variety of new questions and ideas to explore in depth, which were not able to be answered with my studies. I have suggestions for future studies and changes in methods that I would consider if I were to continue these studies.

*Changes in Methods*

For the processing of belowground biomass, I would suggest separating the live and dead material especially when doing an observational study. Some studies eluded that one would be able to separate them easily because the live roots float and the dead roots sink. This would allow for the root to shoot calculations to be closer to the expected range of values. During the growing season in my experiment, any stems that were broken or fallen off were discarded. It might have been beneficial to have the total biomass of the system throughout the study to be able to assess the total production, so I would propose to save all biomass from each mesocosm. I would also suggest running the experiment for a maximum of four to four and a half months. The *P. australis* belowground biomass growth was constrained by the end of six months, in addition to the stems of both species falling over. With regards to the fertilization treatments, the unfertilized mesocosms had significantly stunted growth compared to the fertilized mesocosms which was expected. So, in order to capture more fertilization scenarios, it might be useful to use a low loading rate of fertilization instead of no fertilization. I think a more constant regime should be considered for the levels of salinity, but from other studies' information and my experience I would not recommend raising the levels. Higher levels will just kill off the *P. australis* and leave the *S. cynosuroides* with high stress levels.

*Suggestions of Future Studies*

In order to further understand the rooting depth patterns along a salinity gradient, another observational study along the Nanticoke River would be recommended. It would be interesting to see if the belowground biomass quantified in this study was primarily

dead or alive. Additionally, one could install root bags in the beginning of the growing season to see the amount of new biomass growth within a growing season. The rooting depth patterns and growth rates could be beneficial in predicting and assessing the Nanticoke River's wetlands. Another method to quantify the stress levels within these wetlands would be to take chlorophyll fluorescence measurements of the species growing at these sites. This data could then be corresponded with the other data collected in this area to get an overall stress assessment of the wetland vegetation at the Nanticoke River.

The experimental competition study brought up a wide variety of questions with regards to growth dynamics between species and environmental modification. One of the major questions I have is why *P. australis* growth in terms of RGR and aboveground biomass was higher in mixture than in monoculture. If this facilitation is due to the slight decrease in salinity by the ability of *S. cynosuroides* to uptake and then excrete salt from its leaves, than I would suggest taking sipper measurements of the salinity levels with depth of all mesocosms. The benefit of mixture mesocosms for *P. australis* could be the increased belowground biomass in the upper 10 cm level created by *S. cynosuroides* which allows for greater aeration of the soil thus allowing for higher nutrient uptake. The soil aeration could be measured through the reduction-oxidation potentials within the soil through electrode measurements or the use of IRIS tubes. In order to further examine the effects of *P. australis* growth on accretion and soil retention, one could measure elevation changes within the mesocosms. Due to the flushing and water movement within the mesocosms, the change in elevation would also be effected by the amount of retention and erosion control that the species could provide. These suggestions of further

studies would help clarify some of the interactions occurring between *P. australis* and *S. cynosuroides* and how this applies to the future of the coastal tidal wetland ecosystems.

# APPENDIX A

## *SAS Programs*

A-1 SAS codes to compare the aboveground biomass of each sub-site for the observational study along the Nanticoke River.

```
ODS HTML;
ODS GRAPHICS ON;

OPTIONS LS=124 PS=45 PAGENO=1;

TITLE1 Aboveground biomass Comparison by Sub-site Observational Study
Aug 2009;

data Aboveground;
input Salinity$   rep$ ABbiomass;
datalines;
1a     i      277.3
1a     ii     318.18
1a     iii    324.03
1b     i      347.83
1b     ii     495.72
1b     iii    194.19
1c     i      960.33
1c     ii     946
1c     iii    868.78
2a     i      387.59
2a     ii     542.35
2a     iii    396.81
2b     i      390.8
2b     ii     304.58
2b     iii    453.1
2c     i      1242.78
2c     ii     1049.8
2c     iii    1006.16
3a     i      472.51
3a     ii     323.6
3a     iii    393.27
3b     i      444.6
3b     ii     477.8
3b     iii    528.2
3c     i      274.22
3c     ii     766.82
3c     iii    403.38
4a     i      774.53
4a     ii     844.96
4a     iii    298.34
4b     i      377.3
4b     ii     289.64
4b     iii    405.12
4c     i      497.4
4c     ii     618.59
4c     iii    363.97
5a     i      358.8
5a     ii     572.7
```

```
5a    iii   663.9
5b    i     760.25
5b    ii    596.95
5b    iii   336.7
5c    i     307.34
5c    ii    369.68
5c    iii   401.2
;

PROC MIXED DATA = Aboveground;
CLASS Salinity;
MODEL ABbiomass= Salinity/OUTP=resids ddfm = satterth;
lsmeans Salinity/ adjust=tukey diff=all cl;
ods output lsmeans=lsmean1;
ods listing exclude diffs; ods output diffs=diff1;
ods output tests3=stat1;
run;
quit;

%include "C:\Thesis\Final Paper\RGR\PDMix800.sas";
%pdmix800(diff1,lsmean1,alpha=.05,sort=yes);
quit;

proc plot data=resids vpercent=50;
plot resid*pred/vref=0;
quit;
data resids;
set resids;
aresid=ABS(resid);
run;
proc corr spearman data=resids;
var aresid pred;
quit;
proc univariate data=resids plot normal;
var resid;
quit;
proc print data=lsmean1;
quit;

proc print data=stat1;
quit;
PROC EXPORT DATA= WORK.LSMEAN1
            OUTFILE= "C:\Thesis\NICCR
Research\August09_Sampling\ThesisData\RoottoShoot\Above\aboveSAS.xls"
            DBMS=EXCEL REPLACE;
          NEWFILE=YES;
RUN;
PROC EXPORT DATA= WORK.STAT1
            OUTFILE=  "C:\Thesis\NICCR
Research\August09_Sampling\ThesisData\RoottoShoot\Above\aboveSAS2.xls"
            DBMS=EXCEL REPLACE;
          NEWFILE=YES;
RUN;
ods graphics off;
   ods html close;
   quit;
```

A-2 SAS codes to compare the rooting depth patterns of each sub-site for the observational study along the Nanticoke River.

```
ODS HTML;
ODS GRAPHICS ON;

OPTIONS LS=124 PS=45 PAGENO=1;

TITLE1 Belowground Biomass Depth Comparison Observational Study Aug
2009;

data BelowByDepth;
input Salinity$   rep$ depth biomass;
datalines;
1a    i     5     6.76
1a    i     15    3.45
1a    i     25    2.49
1a    i     35    3.98
1a    i     45    4.58
1a    ii    5     9.01
1a    ii    15    5.64
1a    ii    25    5.49
1a    ii    35    3.99
1a    ii    45    2.57
1a    iii   5     9.83
1a    iii   15    9.21
1a    iii   25    6.06
1a    iii   35    2.52
1a    iii   45    3.53
1b    i     5     5.02
1b    i     15    4.15
1b    i     25    4.35
1b    i     35    3.42
1b    i     45    3.63
1b    ii    5     3.34
1b    ii    15    5.39
1b    ii    25    3.50
1b    ii    35    4.04
1b    ii    45    3.26
1b    iii   5     4.4
1b    iii   15    6.36
1b    iii   25    4.62
1b    iii   35    3.04
1b    iii   45    3.18
1c    i     5     3.61
1c    i     15    3.17
1c    i     25    3.96
1c    i     35    7.28
1c    i     45    0.90
1c    ii    5     7.16
1c    ii    15    7.55
1c    ii    25    5.09
1c    ii    35    2.53
1c    ii    45    2.34
1c    iii   5     7.30
1c    iii   15    6.18
```

| | | | |
|---|---|---|---|
| 1c | iii | 25 | 4.55 |
| 1c | iii | 35 | 5.64 |
| 1c | iii | 45 | 8.75 |
| 2a | i | 5 | 5.78 |
| 2a | i | 15 | 3.75 |
| 2a | i | 25 | 3.12 |
| 2a | i | 35 | 6.10 |
| 2a | i | 45 | 3.62 |
| 2a | ii | 5 | 6.78 |
| 2a | ii | 15 | 6.71 |
| 2a | ii | 25 | 4 |
| 2a | ii | 35 | 9.26 |
| 2a | ii | 45 | 3.89 |
| 2a | iii | 5 | 1.82 |
| 2a | iii | 15 | 3.43 |
| 2a | iii | 25 | 5.01 |
| 2a | iii | 35 | 4.77 |
| 2a | iii | 45 | 6.23 |
| 2b | i | 5 | 2.35 |
| 2b | i | 15 | 1.43 |
| 2b | i | 25 | 0.99 |
| 2b | i | 35 | 1.46 |
| 2b | i | 45 | 4.00 |
| 2b | ii | 5 | 2.34 |
| 2b | ii | 15 | 0.83 |
| 2b | ii | 25 | 1.97 |
| 2b | ii | 35 | 1.00 |
| 2b | ii | 45 | 1.45 |
| 2b | iii | 5 | 10.61 |
| 2b | iii | 15 | 8.28 |
| 2b | iii | 25 | 5.64 |
| 2b | iii | 35 | 5.13 |
| 2b | iii | 45 | 1.90 |
| 2c | i | 5 | 2.01 |
| 2c | i | 15 | 7.55 |
| 2c | i | 25 | 4.82 |
| 2c | i | 35 | 4.08 |
| 2c | i | 45 | 3.06 |
| 2c | ii | 5 | 5.26 |
| 2c | ii | 15 | 4.47 |
| 2c | ii | 25 | 2.74 |
| 2c | ii | 35 | 3.75 |
| 2c | ii | 45 | 2.92 |
| 2c | iii | 5 | 2.10 |
| 2c | iii | 15 | 8.12 |
| 2c | iii | 25 | 8.46 |
| 2c | iii | 35 | 3.26 |
| 2c | iii | 45 | 2.42 |
| 3a | i | 5 | 4.46 |
| 3a | i | 15 | 3.03 |
| 3a | i | 25 | 5.47 |
| 3a | i | 35 | 5.71 |
| 3a | i | 45 | 5.25 |
| 3a | ii | 5 | 0.98 |
| 3a | ii | 15 | 4.52 |
| 3a | ii | 25 | 5.51 |
| 3a | ii | 35 | 8.91 |

| | | | |
|---|---|---|---|
| 3a | ii | 45 | 8.30 |
| 3a | iii | 5 | 2.69 |
| 3a | iii | 15 | 5.16 |
| 3a | iii | 25 | 5.29 |
| 3a | iii | 35 | 20.72 |
| 3a | iii | 45 | 13.23 |
| 3b | i | 5 | 0.93 |
| 3b | i | 15 | 1.15 |
| 3b | i | 25 | 1.17 |
| 3b | i | 35 | 0.71 |
| 3b | i | 45 | 1.09 |
| 3b | ii | 5 | 1.89 |
| 3b | ii | 15 | 4.19 |
| 3b | ii | 25 | 7.76 |
| 3b | ii | 35 | 2.14 |
| 3b | ii | 45 | 1.29 |
| 3b | iii | 5 | 1.73 |
| 3b | iii | 15 | 0.53 |
| 3b | iii | 25 | 3.69 |
| 3b | iii | 35 | 1.25 |
| 3b | iii | 45 | 0.63 |
| 3c | i | 5 | 2.35 |
| 3c | i | 15 | 6.86 |
| 3c | i | 25 | 4.17 |
| 3c | i | 35 | 2.77 |
| 3c | i | 45 | 2.72 |
| 3c | ii | 5 | 2.22 |
| 3c | ii | 15 | 1.62 |
| 3c | ii | 25 | 4.28 |
| 3c | ii | 35 | 3.65 |
| 3c | ii | 45 | 1.83 |
| 3c | iii | 5 | 1.65 |
| 3c | iii | 15 | 2.2 |
| 3c | iii | 25 | 1.88 |
| 3c | iii | 35 | 1.64 |
| 3c | iii | 45 | 1.95 |
| 4a | i | 5 | 5.42 |
| 4a | i | 15 | 5.21 |
| 4a | i | 25 | 10.75 |
| 4a | i | 35 | 7.37 |
| 4a | i | 45 | 5.20 |
| 4a | ii | 5 | 6.16 |
| 4a | ii | 15 | 4.71 |
| 4a | ii | 25 | 5.10 |
| 4a | ii | 35 | 6.37 |
| 4a | ii | 45 | 2.13 |
| 4a | iii | 5 | 2.23 |
| 4a | iii | 15 | 7.36 |
| 4a | iii | 25 | 10.84 |
| 4a | iii | 35 | 4.02 |
| 4a | iii | 45 | 3.12 |
| 4b | i | 5 | 6.85 |
| 4b | i | 15 | 6.88 |
| 4b | i | 25 | 2.73 |
| 4b | i | 35 | 5.42 |
| 4b | i | 45 | 5.50 |
| 4b | ii | 5 | 1.12 |

| | | | |
|---|---|---|---|
| 4b | ii | 15 | 1.88 |
| 4b | ii | 25 | 4.51 |
| 4b | ii | 35 | 3.32 |
| 4b | ii | 45 | 2.98 |
| 4b | iii | 5 | 1.85 |
| 4b | iii | 15 | 0.75 |
| 4b | iii | 25 | 0.39 |
| 4b | iii | 35 | 1.00 |
| 4b | iii | 45 | 1.95 |
| 4c | i | 5 | 8.61 |
| 4c | i | 15 | 7.47 |
| 4c | i | 25 | 11.17 |
| 4c | i | 35 | 9.47 |
| 4c | i | 45 | 3.37 |
| 4c | ii | 5 | 6.40 |
| 4c | ii | 15 | 7.61 |
| 4c | ii | 25 | 7.23 |
| 4c | ii | 35 | 10.09 |
| 4c | ii | 45 | 7.77 |
| 4c | iii | 5 | 5.05 |
| 4c | iii | 15 | 10.41 |
| 4c | iii | 25 | 7.44 |
| 4c | iii | 35 | 7.69 |
| 4c | iii | 45 | 4.95 |
| 5a | i | 5 | 8.18 |
| 5a | i | 15 | 7.17 |
| 5a | i | 25 | 5.09 |
| 5a | i | 35 | 1.02 |
| 5a | i | 45 | 0.99 |
| 5a | ii | 5 | 3.98 |
| 5a | ii | 15 | 6.27 |
| 5a | ii | 25 | 3.14 |
| 5a | ii | 35 | 2.39 |
| 5a | ii | 45 | 2.05 |
| 5a | iii | 5 | 1.58 |
| 5a | iii | 15 | 1.13 |
| 5a | iii | 25 | 2.05 |
| 5a | iii | 35 | 2.00 |
| 5a | iii | 45 | 0.77 |
| 5b | i | 5 | 1.24 |
| 5b | i | 15 | 2.42 |
| 5b | i | 25 | 2.9 |
| 5b | i | 35 | 2.34 |
| 5b | i | 45 | 3.15 |
| 5b | ii | 5 | 2.39 |
| 5b | ii | 15 | 4.63 |
| 5b | ii | 25 | 4.79 |
| 5b | ii | 35 | 5.09 |
| 5b | ii | 45 | 1.53 |
| 5b | iii | 5 | 3.87 |
| 5b | iii | 15 | 5.40 |
| 5b | iii | 25 | 3.51 |
| 5b | iii | 35 | 3.43 |
| 5b | iii | 45 | 1.45 |
| 5c | i | 5 | 3.87 |
| 5c | i | 15 | 3.78 |
| 5c | i | 25 | 3.47 |

```
5c      i       35      2.6
5c      i       45      3.07
5c      ii      5       1.6
5c      ii      15      1.70
5c      ii      25      3.44
5c      ii      35      2.80
5c      ii      45      2.19
5c      iii     5       3
5c      iii     15      5.50
5c      iii     25      4.87
5c      iii     35      3.43
5c      iii     45      2.63
;

PROC MIXED DATA = BelowByDepth;
CLASS Salinity depth;
MODEL biomass= Salinity|depth/OUTP=resids ddfm = satterth;
*repeated / group = depth;
lsmeans Salinity|depth/ adjust=tukey diff=all cl;
ods output lsmeans=lsmean1;
ods listing exclude diffs; ods output diffs=diff1;
ods output tests3=stat1;
run;
quit;

%include "C:\Thesis\Final Paper\RGR\PDMix800.sas";
%pdmix800(diff1,lsmean1,alpha=.05,sort=yes);
quit;

proc plot data=resids vpercent=50;
plot resid*pred/vref=0;
quit;
data resids;
set resids;
aresid=ABS(resid);
run;
proc corr spearman data=resids;
var aresid pred;
quit;
proc univariate data=resids plot normal;
var resid;
quit;
proc print data=lsmean1;
quit;

proc print data=stat1;
quit;
PROC EXPORT DATA= WORK.LSMEAN1
            OUTFILE= "C:\Thesis\NICCR
Research\August09_Sampling\ThesisData\Rooting Depth\depthSAS.xls"
            DBMS=EXCEL REPLACE;
          NEWFILE=YES;
RUN;
PROC EXPORT DATA= WORK.STAT1
            OUTFILE=  "C:\Thesis\NICCR
Research\August09_Sampling\ThesisData\Rooting Depth\depthSAS2.xls"
            DBMS=EXCEL REPLACE;
```

```
          NEWFILE=YES;
RUN;
ods graphics off;
   ods html close;
   quit;
```

A-3 SAS codes to compare the rooting depth patterns of *Phragmites australis* in varying levels of salinity, nitrogen, and competition (S indicates Fertilized, N indicates Unfertilized)

```
ODS HTML;
ODS GRAPHICS ON;

OPTIONS LS=124 PS=45 PAGENO=1;

TITLE1 Belowground Biomass Depth Comparison Phragmites australis 2010;

data BelowDepth_Phrag;
input Planting$ Salinity$     Nitrogen$ depth block   biomass_phrag;
datalines;
P      4      S      5      1      175.9
P      4      S      15     1      126.3
P      4      S      25     1      111.4
P      4      S      35     1      109.4
P      16     S      5      1      147.8
P      16     S      15     1      45.8
P      16     S      25     1      99
P      16     S      35     1      131.6
P      4      N      5      1      32.6
P      4      N      15     1      53.4
P      4      N      25     1      38.1
P      4      N      35     1      60.2
P      0      S      5      1      164.1
P      0      S      15     1      141.5
P      0      S      25     1      113.4
P      0      S      35     1      81.3
P      8      S      5      1      136.9
P      8      S      15     1      107.9
P      8      S      25     1      130.3
P      8      S      35     1      136.3
P      8      N      5      1      49.5
P      8      N      15     1      47.5
P      8      N      25     1      15.7
P      8      N      35     1      11.8
P      0      N      5      1      64.9
P      0      N      15     1      45.5
P      0      N      25     1      42.8
P      0      N      35     1      52.9
P      16     N      5      1      30.8
P      16     N      15     1      50.6
P      16     N      25     1      22.3
P      16     N      35     1      46.8
P      0      N      5      2      44.7
P      0      N      15     2      58.9
P      0      N      25     2      38.6
P      0      N      35     2      60.5
P      4      S      5      2      202.1
P      4      S      15     2      107.4
P      4      S      25     2      116.6
P      4      S      35     2      149.8
P      4      N      5      2      46.1
P      4      N      15     2      53.3
```

```
P       4       N       25      2       42.4
P       4       N       35      2       60.5
P       8       S       5       2       192.8
P       8       S       15      2       219.1
P       8       S       25      2       109.3
P       8       S       35      2       108.9
P       0       S       5       2       167.1
P       0       S       15      2       113.4
P       0       S       25      2       96.2
P       0       S       35      2       69.4
P       8       N       5       2       50.2
P       8       N       15      2       44.3
P       8       N       25      2       57.5
P       8       N       35      2       37.7
P       16      S       5       2       26.9
P       16      S       15      2       92.3
P       16      S       25      2       37.5
P       16      S       35      2       31.2
P       16      N       5       2       41
P       16      N       15      2       43.4
P       16      N       25      2       30.7
P       16      N       35      2       33.1
P       8       S       5       3       212.4
P       8       S       15      3       129.8
P       8       S       25      3       110.5
P       8       S       35      3       66
P       8       N       5       3       43.3
P       8       N       15      3       79.9
P       8       N       25      3       52.1
P       8       N       35      3       44.4
P       16      N       5       3       75.3
P       16      N       15      3       56.9
P       16      N       25      3       47.9
P       16      N       35      3       126.6
P       4       N       5       3       75.7
P       4       N       15      3       66.4
P       4       N       25      3       56.8
P       4       N       35      3       58.2
P       16      S       5       3       112.5
P       16      S       15      3       134.1
P       16      S       25      3       131.6
P       16      S       35      3       48.6
P       0       S       5       3       135.2
P       0       S       15      3       135.6
P       0       S       25      3       98.9
P       0       S       35      3       97
P       0       N       5       3       49.1
P       0       N       15      3       56.4
P       0       N       25      3       39.3
P       0       N       35      3       44
P       4       S       5       3       102.5
P       4       S       15      3       146.5
P       4       S       25      3       78.6
P       4       S       35      3       136.9
;

PROC MIXED DATA = BelowDepth_Phrag;
```

```
CLASS Salinity Nitrogen depth block;
MODEL biomass_Phrag= Salinity|Nitrogen|depth block/OUTP=resids ddfm =
satterth;
random block;
lsmeans Salinity|Nitrogen|depth/ adjust=tukey diff=all cl;
ods output lsmeans=lsmean1;
ods listing exclude diffs; ods output diffs=diff1;
ods output tests3=stat1;
run;
quit;

%include "C:\Thesis\Final Paper\RGR\PDMix800.sas";
%pdmix800(diff1,lsmean1,alpha=.05,sort=yes);
quit;

proc plot data=resids vpercent=50;
plot resid*pred/vref=0;
quit;
data resids;
set resids;
aresid=ABS(resid);
run;
proc corr spearman data=resids;
var aresid pred;
quit;
proc univariate data=resids plot normal;
var resid;
quit;
proc print data=lsmean1;
quit;

proc print data=stat1;
quit;
PROC EXPORT DATA= WORK.LSMEAN1
            OUTFILE= "C:\Thesis\Final Paper\Below
Biomass\belowdepthSAS_Phrag.XLS"
            DBMS=EXCEL REPLACE;
          NEWFILE=YES;
RUN;
PROC EXPORT DATA= WORK.STAT1
            OUTFILE= "C:\Thesis\Final Paper\Below
Biomass\belowdepthSAS_Phrag2.XLS"
            DBMS=EXCEL REPLACE;
          NEWFILE=YES;
RUN;
ods graphics off;
   ods html close;
   quit;
```

A-4 SAS codes to compare the relative growth rates (RGR) of *Phragmites australis* in varying levels of salinity, nitrogen, and competition. (P: Monoculture, B: Mixed, S: Fertilized, N: Unfertilized)

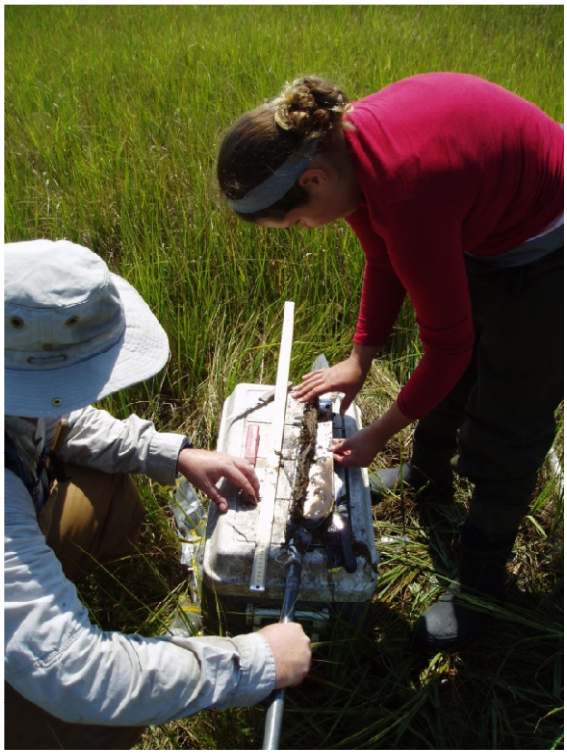
```
ODS HTML;
ODS GRAPHICS ON;

OPTIONS LS=124 PS=45 PAGENO=1;

TITLE1 RGR from June to July Phragmites australis 2010;

data RGR_juneJuly;
input block Planting$ Salinity$     Nitrogen$   RGR_phrag;
datalines;
1      P      4      S      0.049934329
1      P      16     S      0.046719593
1      B      4      N      0.040792514
1      B      8      N      0.044583473
1      P      4      N      0.038455736
1      P      0      S      0.052854242
1      B      16     S      0.050683014
1      P      8      S      0.049867558
1      B      0      N      0.042839941
1      B      0      S      0.051530817
1      P      8      N      0.03735304
1      P      0      N      0.042348752
1      B      8      S      0.044944981
1      P      16     N      0.032798313
1      B      4      S      0.0392885
1      B      16     N      0.020266312
2      B      0      S      0.054404311
2      P      0      N      0.039841304
2      B      8      S      0.048174563
2      P      4      S      0.053278436
2      B      16     N      0.042413666
2      P      4      N      0.032330006
2      P      8      S      0.043247532
2      B      0      N      0.030999653
2      P      0      S      0.046122893
2      B      4      S      0.05184569
2      B      4      N      0.047872206
2      B      8      N      0.029412973
2      P      8      N      0.033108392
2      P      16     S      0.043134569
2      P      16     N      0.034892907
2      B      16     S      0.041642419
3      B      0      N      0.049967136
3      B      8      N      0.04132303
3      P      8      S      0.041945717
3      B      16     N      0.037761515
3      P      8      N      0.027774532
3      P      16     N      0.031766892
3      P      4      N      0.03394602
3      B      4      N      0.031022649
3      P      16     S      0.049505775
3      P      0      S      0.046974776
```

```
3       P       0       N       0.034186238
3       B       4       S       0.039157866
3       B       16      S       0.049469737
3       P       4       S       0.040808583
3       B       0       S       0.057019869
3       B       8       S       0.051545993
;

PROC MIXED DATA = RGR_juneJuly;
CLASS Salinity Nitrogen Planting block;
MODEL RGR_phrag= Planting|Salinity|Nitrogen block/OUTP=resids ddfm =
satterth;
random block;
lsmeans Planting|Salinity|Nitrogen/ adjust=tukey diff=all cl;
ods output lsmeans=lsmean1;
ods listing exclude diffs; ods output diffs=diff1;
ods output tests3=stat1;
run;
quit;

%include 'C:\Thesis\Final Paper\RGR\PDMix800.sas';
%pdmix800(diff1,lsmean1,alpha=.05,sort=yes);
quit;

proc plot data=resids vpercent=50;
plot resid*pred/vref=0;
quit;
data resids;
set resids;
aresid=ABS(resid);
run;
proc corr spearman data=resids;
var aresid pred;
quit;
proc univariate data=resids plot normal;
var resid;
quit;
proc print data=lsmean1;
quit;

proc print data=stat1;
quit;
PROC EXPORT DATA= WORK.LSMEAN1
            OUTFILE= "C:\Thesis\Final
Paper\RGR\JuneJuly\Phrag_RGRJuneJuly_SAS.XLS"
            DBMS=EXCEL REPLACE;
          NEWFILE=YES;
RUN;
PROC EXPORT DATA= WORK.STAT1
            OUTFILE= "C:\Thesis\Final
Paper\RGR\JuneJuly\Phrag_RGRJuneJuly_SAS2.XLS"
            DBMS=EXCEL REPLACE;
          NEWFILE=YES;
RUN;
ods graphics off;
   ods html close;
   quit;
```

# APPENDIX B

*Pictures of Observational Study*

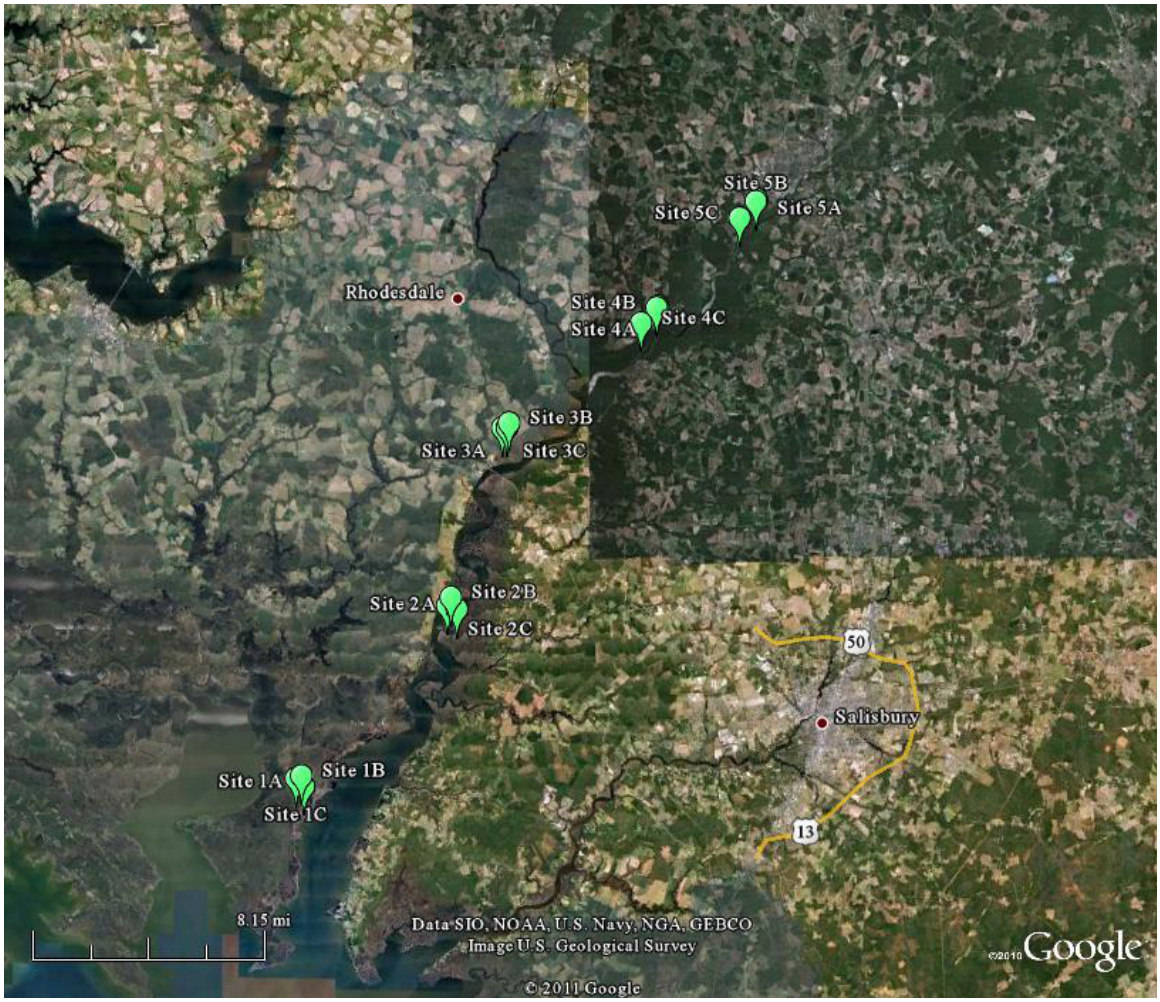
B- 1: Aboveground biomass harvesting within the 1-$m^2$ quadrat.



B- 2 Belowground biomass harvesting using the peat corer.

# APPENDIX C

*Additional Figures for the Observational Study*

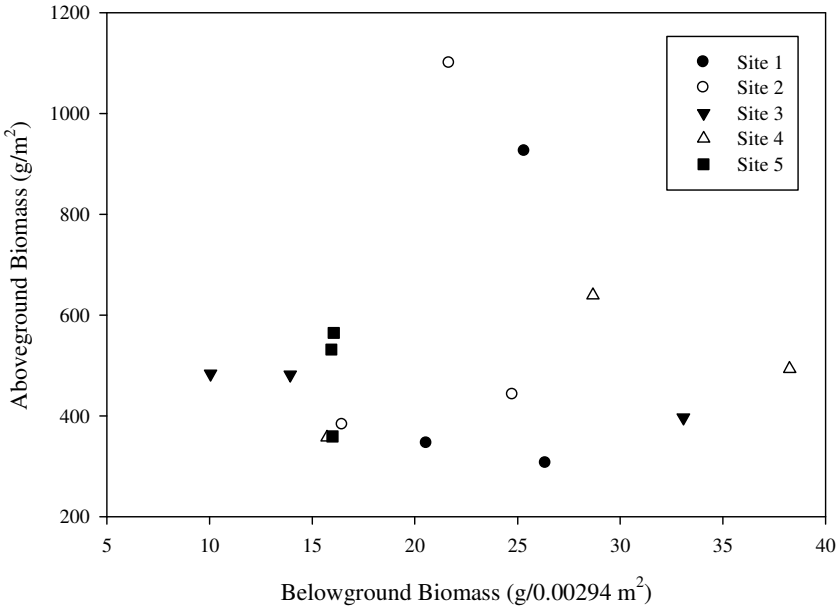



C- 1 Map depicting the locations of the sub-sites along the Nanticoke River in eastern Maryland. Map courtesy of Google Earth.

C- 2 List of all species seen in the observational study along the Nanticoke River by site.

| Site | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | | 2 | | | 3 | | | 4 | | | 5 | | | | | |
| A | B | C | A | B | C | A | B | C | A | B | C | A | B | C | **Abbrev.** | **Latin Name** |
| | | | | | | | | | X | X | X | X | | X | Aco cal | *Acorus calamus* L. |
| | | | | | | | | X | | | | | X | X | Bid fro | *Bidens frondosa* L. |
| | | | | | | | | X | | | | | | | Bid lae | *Bidens laevis* (L.) Britton, Sterns & Poggenb. |
| | | | | | | | | | X | | | | | | Bid mit | *Bidens mitis* (Michx.) Sherff |
| | | | | | | | | | | | X | | | | Boe cyl | *Boehmeria cylindrica* (L.) Sw. |
| | | | | | | | | | | | X | | | | Car lac | *Carex lacustris* Willd. |
| | | | | | | | | X | | | | | | | Car sp. | *Carex sp.* |
| | | | | | | | | | | | X | | | | Car tri | *Carex tribuloides* Wahlenb. |
| | | | | | | | | | X | | | | | | Cic mac | *Cicuta maculata* L. |
| | | | | | | | | | X | | X | | | X | Cin aru | *Cinna arundinacea* L. |
| | | | | | | | | | | | X | | X | | Cus eur | *Cuscuta europaea* L. |
| X | X | | X | | | | | | | | | | | | Dis spi | *Distichlis spicata* (L.) Greene |
| | | | | X | | X | | | | | | | | | Ele obt | *Eleocharis obtusa* (Willd.) Schult. |
| | X | | | | | | | | | | | | | | Fin cas | *Fimbristylis castanea* (Michx.) Vahl |
| | | | | | | X | | | | | X | | | X | Gal tin | *Galium tinctorium* (L.) Scop. |
| | | | | X | | | | X | X | | | | | | Hib mos | *Hibiscus moscheutos* L. |
| | | | | | | X | | | X | X | X | | X | X | Imp cap | *Impatiens capensis* Meerb. |
| | | | X | | | | | | | | | | | | Iva fru | *Iva frutescens* L. |
| | | X | | | | | | | | | | | | | Jun roe | *Juncus roemerianus* Scheele |
| | | | X | | | | | | | | | | | | Kos vir | *Kosteletzkya virginica* (L.) C. Presl ex A. Gray |
| | | | | | | X | | | X | | X | | X | X | Lee ory | *Leersia oryzoides* (L.) Sw. |
| | | | | | | | | | | | | | | X | Men arv | *Mentha arvensis* L. |
| | | | | | | | | | | | | | X | | Mur kei | *Murdannia keisak* (Hassk.) Hand.-Maz. |
| | | | | | | X | X | X | X | X | X | X | X | X | Pel vir | *Peltandra virginica* (L.) Schott |
| | | | | | | X | | | | | | | | | Phr aus | *Phragmites australis* (Cav.) Trin. ex Steud. |
| X | | | X | | | | | | | | | | | | Plu pir | *Pluchea purpurascens* (Sw.) DC. |
| | | | | | | X | X | X | X | X | X | X | X | X | Pol ari | *Polygonum arifolium* L. |
| | | | | | | X | | | | | | | | | Pol pens | *Polygonum pensylvanicum* L. |

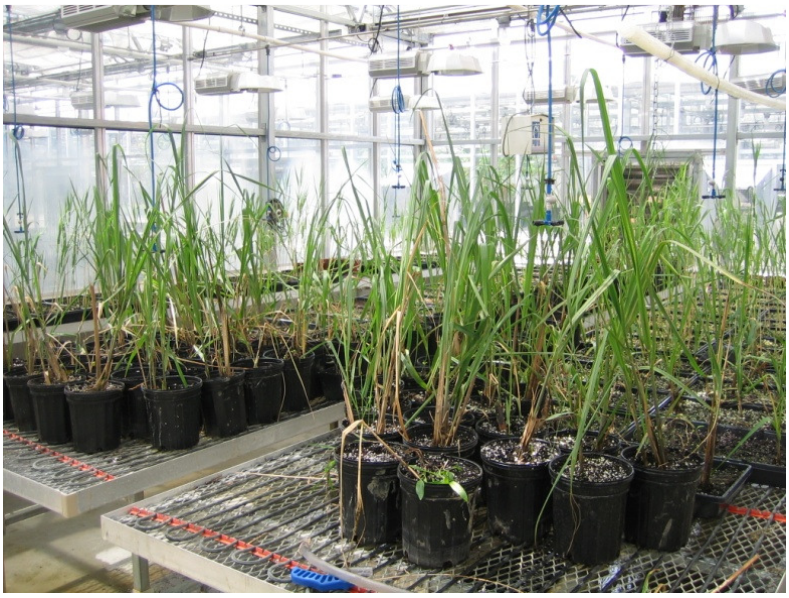
| Site | | | | | | | | | | | | | | | Abbrev. | Latin Name |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | | 2 | | | 3 | | | 4 | | | 5 | | | | |
| A | B | C | A | B | C | A | B | C | A | B | C | A | B | C | | |
| | | | X | X | X | | | X | | | | | X | | Pol pun | *Polygonum punctatum* Elliot. |
| | | | | | | | | | | X | | | | X | Sag lat | *Sagittaria latifolia* Willd. |
| | | | | | | X | | | | | | | | | Sch ame | *Schoenoplectus americanus* (Pers.) Volkart ex Schinz & R. Keller |
| | | | | | | X | | | X | X | X | | | | Sch flu | *Schoenoplectus fluviatilis* (Torr.) M.T. Strong |
| | | | X | X | X | | | | | | X | | | | Sch rob | *Schoenoplectus robustus* (Pursh) M.T. Strong |
| X | X | | X | X | X | | | | | | | | | | Spa alt | *Spartina alterniflora* Loisel. |
| | | | X | X | X | | | | | | | | | | Spa cyn | *Spartina cynosuroides* (L.) Roth |
| X | X | | X | | | | | | | | | | | | Spa pat | *Spartina patens* (Aiton) Muhl. |
| | | | | | | X | | | X | | X | X | X | X | Sym pun | *Symphyotrichum puniceum* (L.) A. Löve & D. Löve |
| | | | | | | X | | | | | | | | | Teu can | *Teucrium canadense* L. |
| | | | | | | X | X | | X | | X | | X | | Typ lat | *Typha latifolia* L. |
| | | | | | | X | | X | | | X | | | | Typ sp | *Typha sp.* |
| | | | | | | | | | | | | X | X | X | Ziz aqu | *Zizania aquatica* L. |



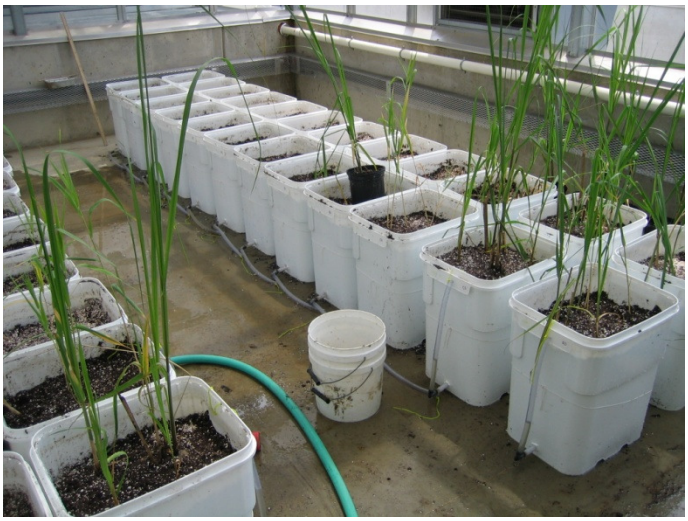

C- 3 Comparison of the aboveground biomass and belowground biomass (from the three cores) at each sample plot estimated over each sub-site. Points represent each sub-site within each site. Sites 1 and 2 are classified as brackish wetlands, sites 3 and 4 are classified as oligohaline wetlands, and Site 5 is a tidal fresh wetland environment. Values are least square means.

# APPENDIX D

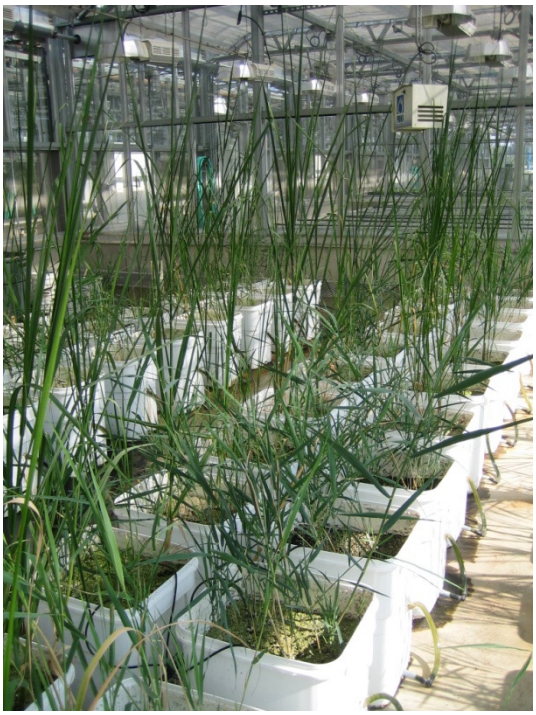
*Pictures of the Experimental Greenhouse Study*



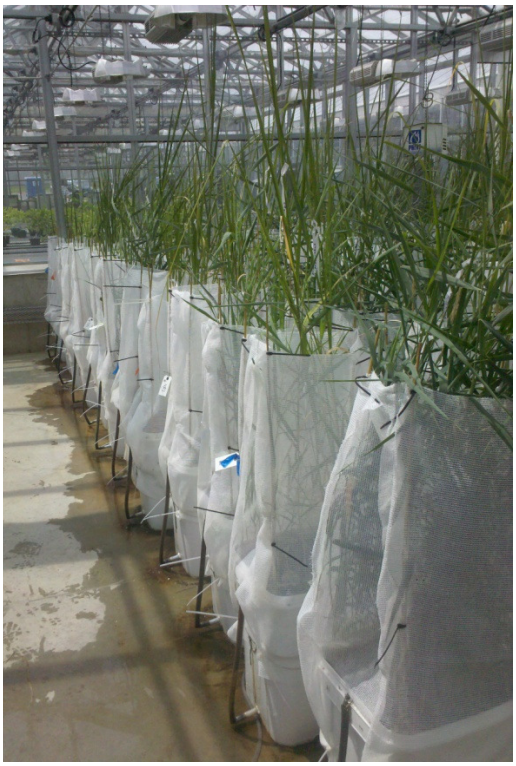
D- 1 Rhizomes and shoots collected from Clyde Watson Boating Ramp in the misting room in the University of Maryland Research Greenhouse.



D- 2 The shoots being planted in the mesocosms planting showing the original planting schemes.



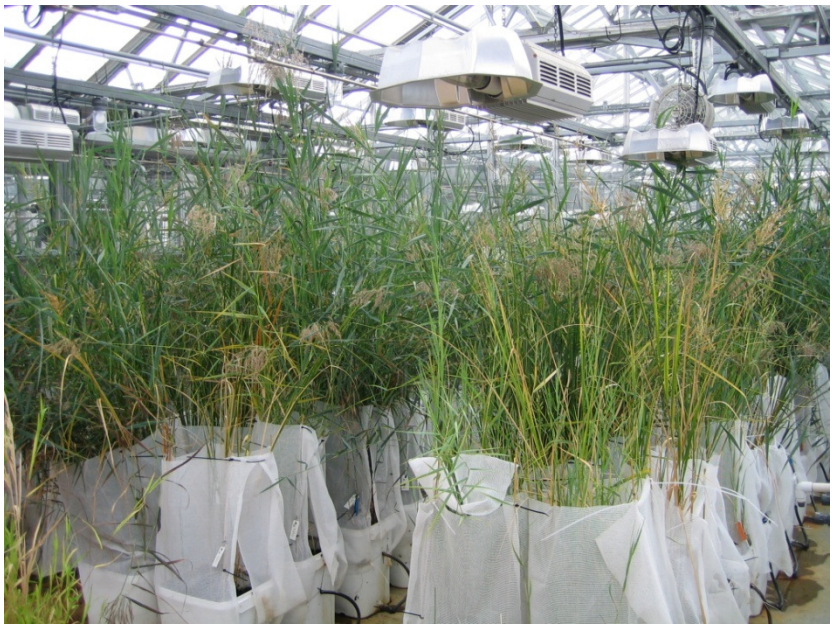
D- 3 The growth of the mesocosms after one month, late June 2010, showing the mesocosms before the shade cloths were installed.



D- 4 The growth of the mesocosms after two months, late July 2010, showing the installation of the shade cloths.

D- 5 Stem height measurements in late September 2010 of all mesocosms.

D- 6 Depiction of the height of some of the samples in early December 2010 and the fire hazard created by the greenhouse supplemental lighting.



D- 7 Growth of mesocosms over the six month experiment period. Picture was taken right before harvesting in late December 2010.

D- 8 Stem height measurements during harvesting were done after stems were removed from the mesocosms.

D- 9 Monoculture *Phragmites australis* mesocosm prior to processing of biomass.

D- 10 Monoculture belowground biomass processing by depth.

D- 11 Depicting one 10-cm depth section for the belowground biomass monoculture processing by depth.

D- 12 Depiction of the large amounts of belowground biomass growth in a fertilized, monoculture mesocosm of *Phragmites australis*.

D- 13 Depiction of the process of separating the belowground biomass by species in the mixed mesocosms.

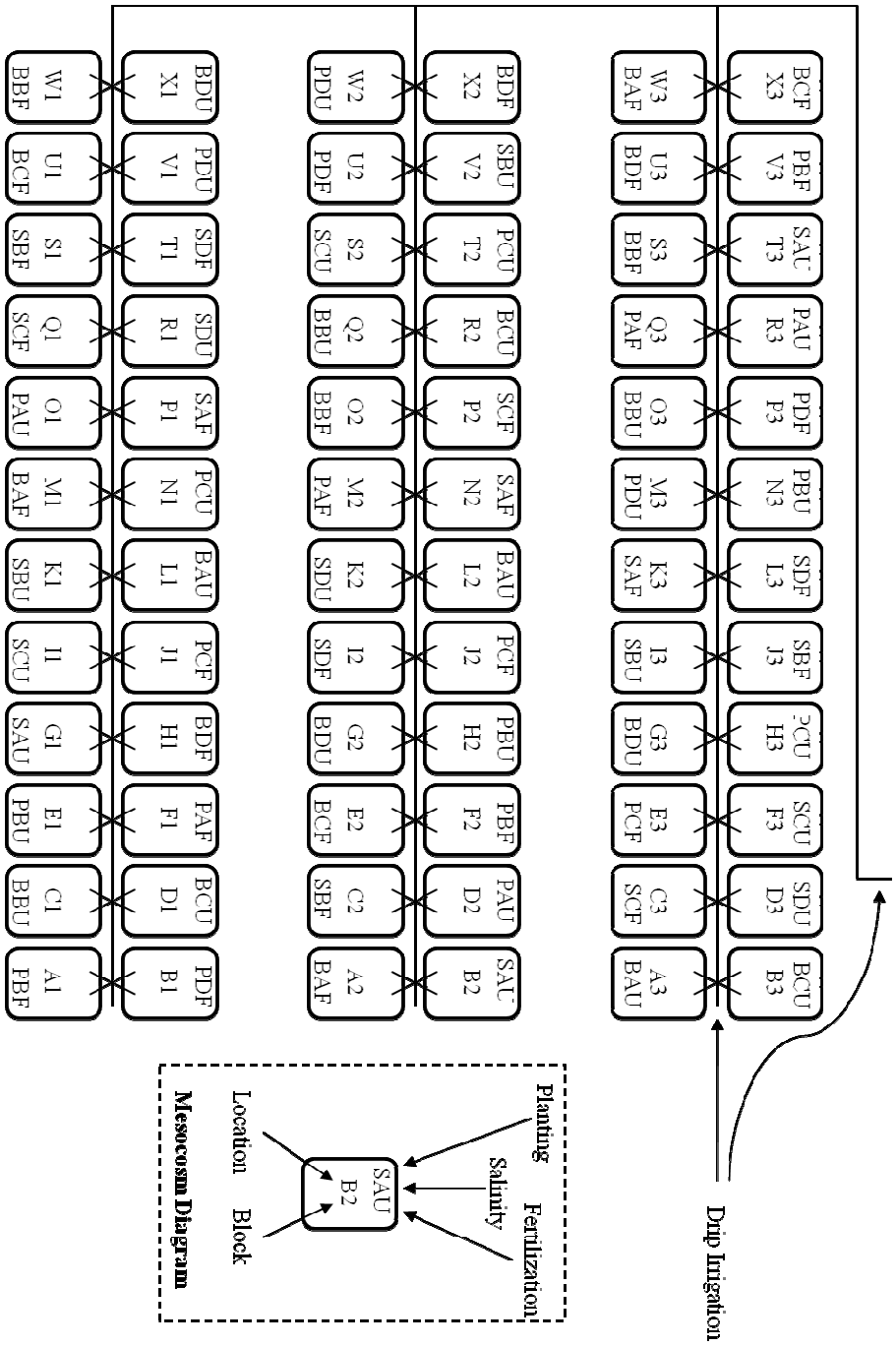
D- 14 Visual depiction of the belowground biomass growth in mixture. Region gestured was the growth of *Spartina cynosuroides* and the remaining was *Phragmites australis* growth.

D- 15 Visual depiction of the differences in belowground biomass growth between *Spartina cynosuroides* and *Phragmites australis*. *S. cynosuroides* was shown on the left and *P. australis* was on the right.

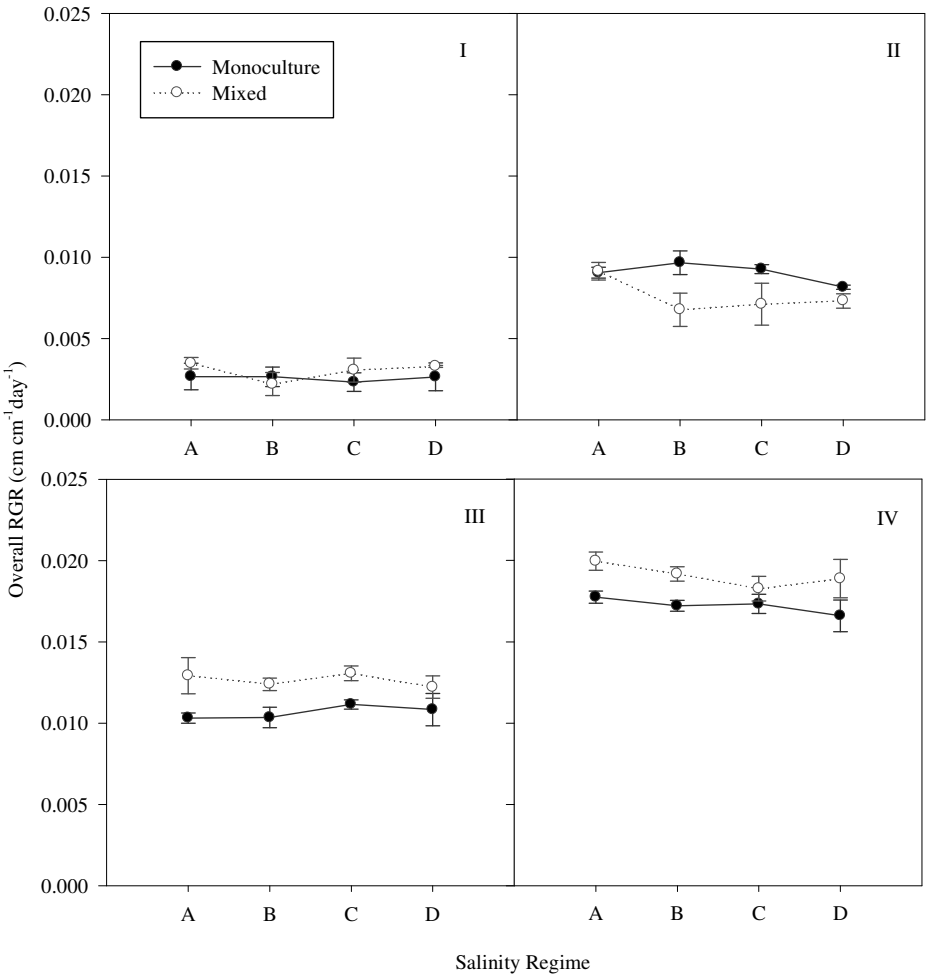
D- 16 Belowground biomass processing which was done to ensure all growing medium was removed from the biomass.

# APPENDIX E

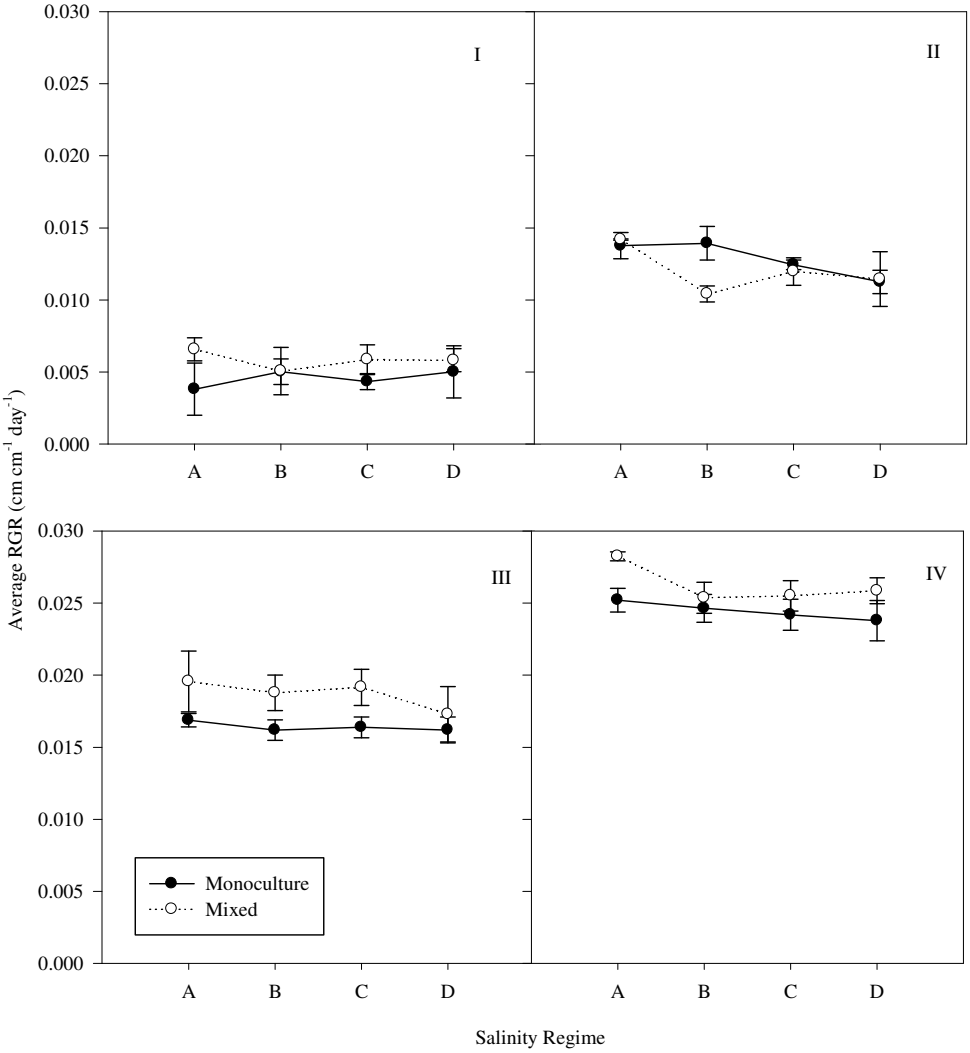
*Additional Figures for the Experimental Greenhouse Study*



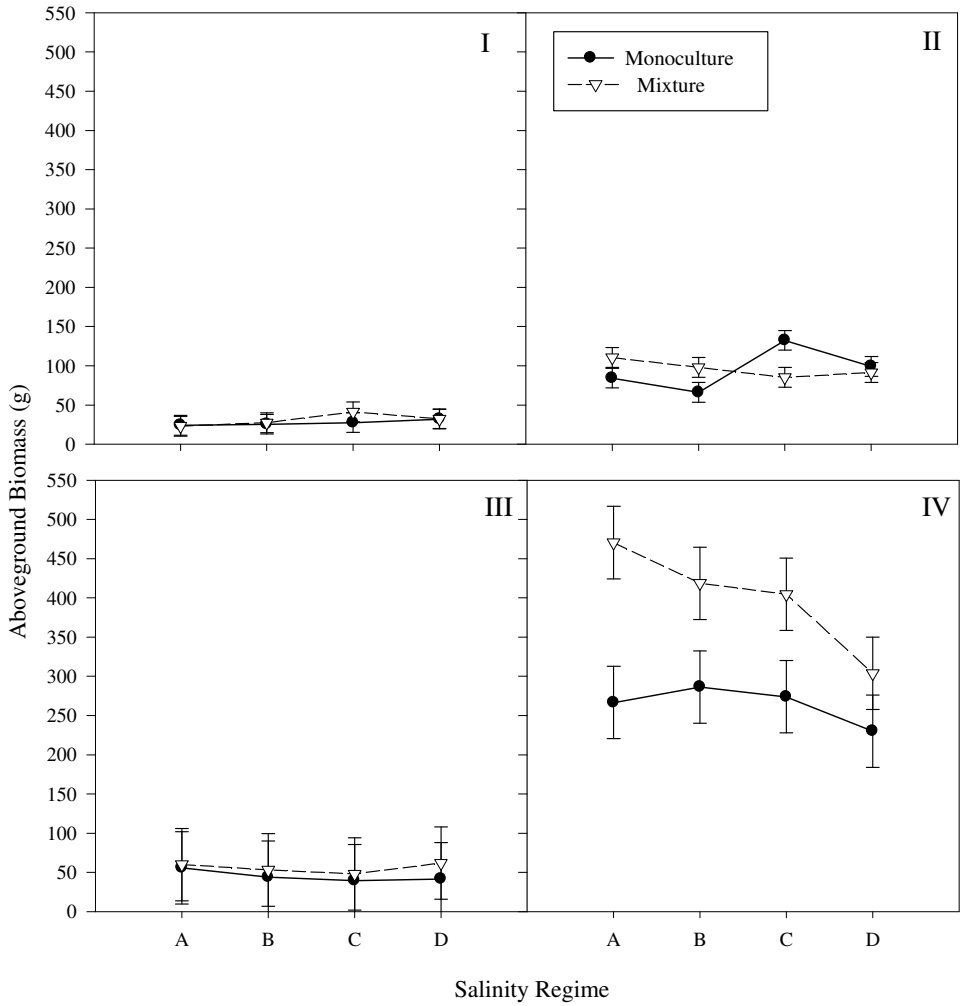

E-1 Mesocosm layout and treatments within the Research Greenhouse at University of Maryland College Park. Drip irrigation shown was used to ensure that the mesocosms were kept at a constant water level throughout the experiment. Codes for treatments are as follows: Planting: *Phragmites australis* monoculture (P), *Spartina cynosuroides* monoculture (S), mixed (B); Salinity: (by increasing salinity) Level A (A), Level B (B), Level C (C), Level D (D); Fertilization: Fertilized (F), Unfertilized (U).



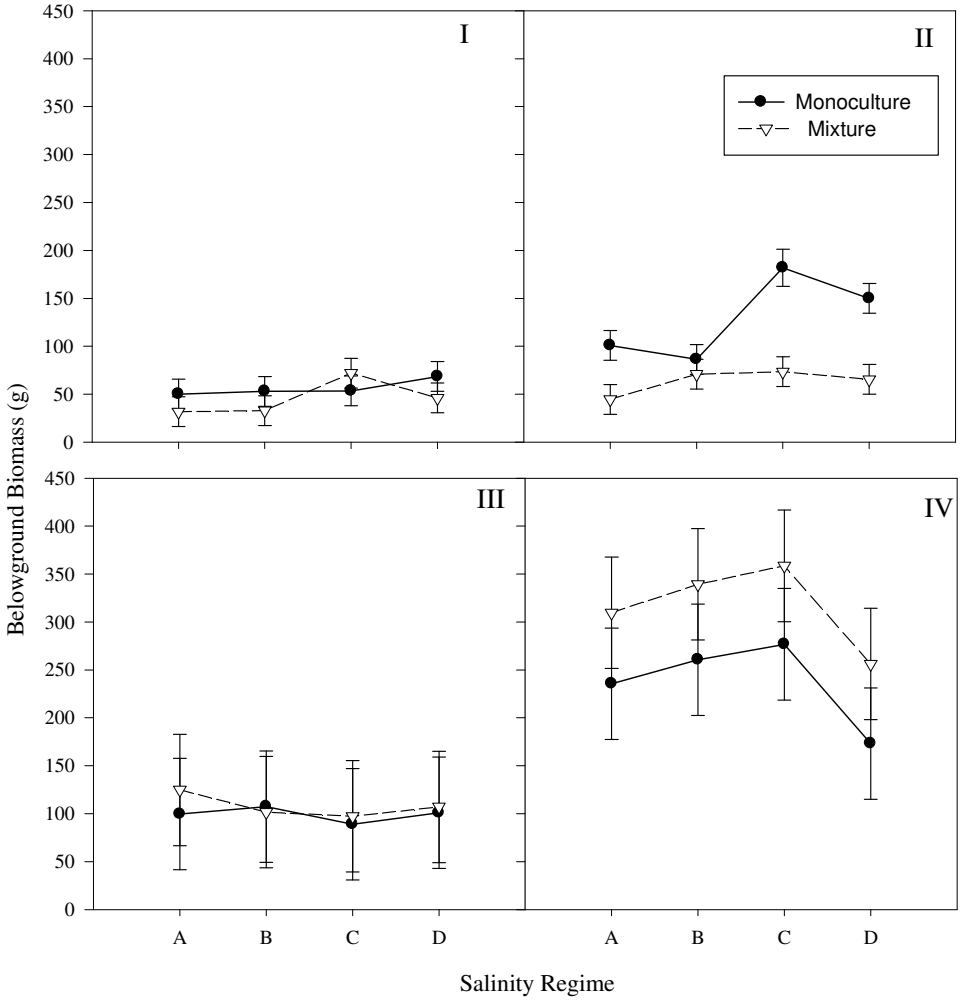

E-2 Comparison of competition effects on overall relative growth rates based on stem height measurements from June and December 2010 in different treatments of salinity and fertilization. I. *S. cynosuroides* with no nitrogen additions. II. *S. cynosuroides* with nitrogen additions. III. *P. australis* with no nitrogen additions. IV. *P. australis* with nitrogen additions. Plotted values are least square means. Error bars shown are standard error.



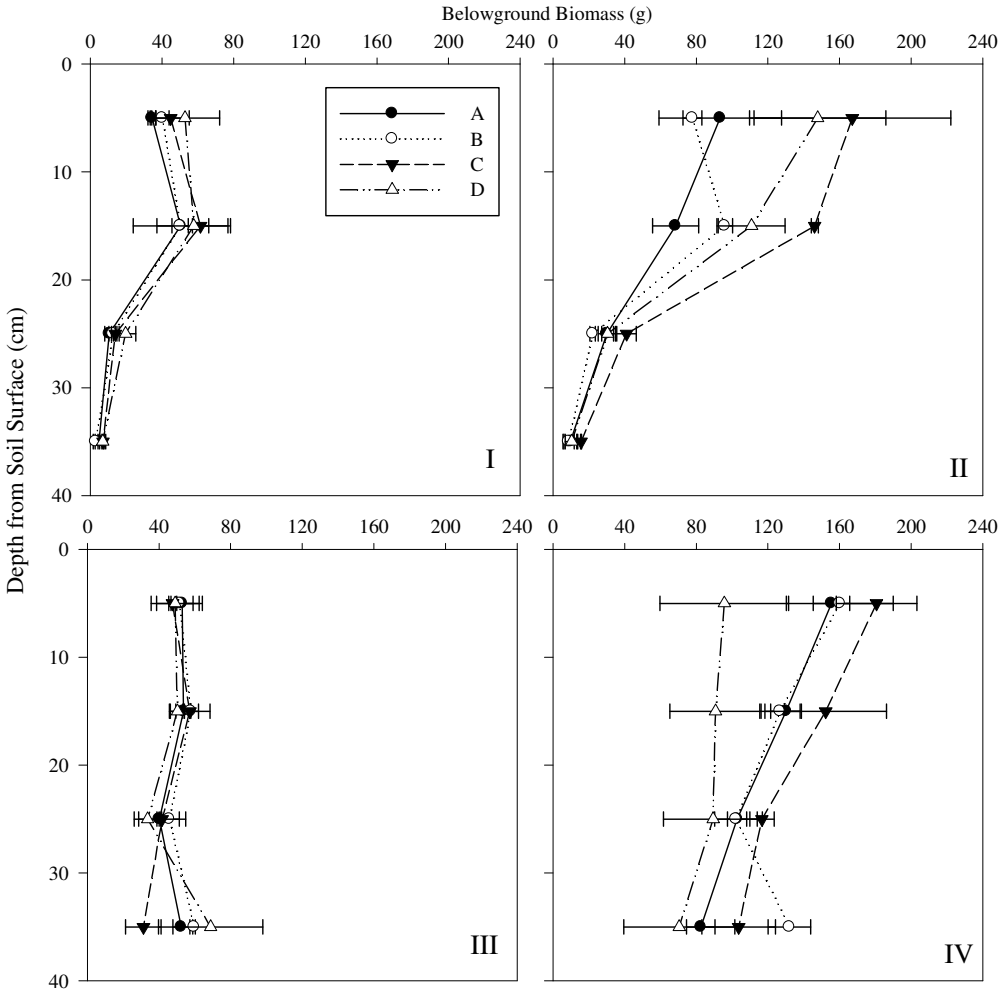

E-3 Comparison of competition effects on average relative growth rates based on stem height measurements from June, July, September and December 2010 in different treatments of salinity and fertilization. I. *S. cynosuroides* with no nitrogen additions. II. *S. cynosuroides* with nitrogen additions. III. *P. australis* with no nitrogen additions. IV. *P. australis* with nitrogen additions. Plotted values are least square means. Error bars shown are standard error.



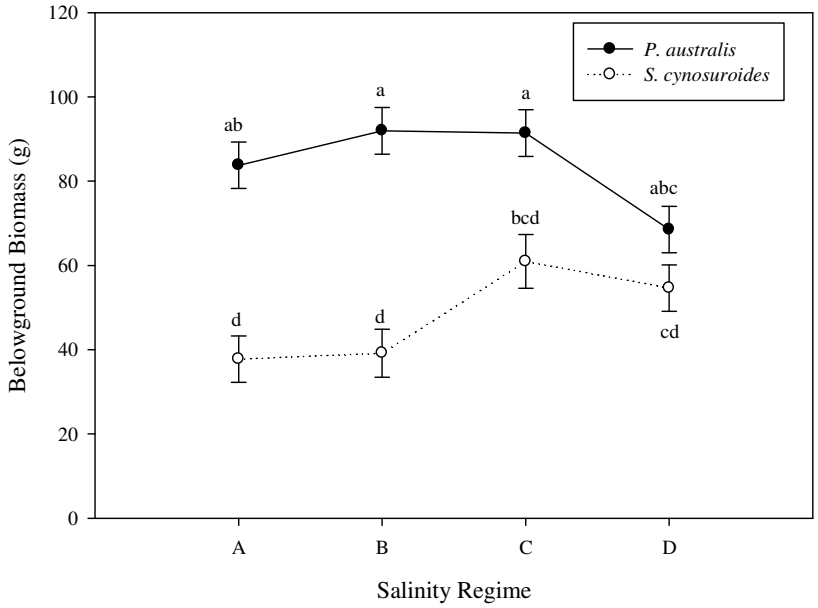

E-4 Comparison of competition effects on aboveground biomass collected in December 2010 in different treatments of salinity and fertilization. I. *S. cynosuroides* with no nitrogen additions. II. *S. cynosuroides* with nitrogen additions. III. *P. australis* with no nitrogen additions. IV. *P. australis* with nitrogen additions. Plotted values are least square means. Error bars shown are standard error.



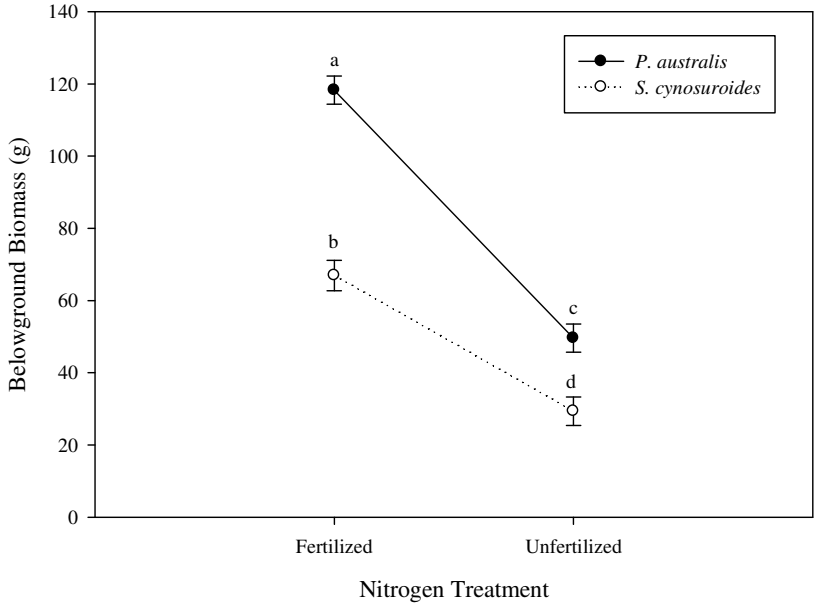

E-5 Comparison of competition effects on belowground biomass collected in December 2010 in different treatments of salinity and fertilization. I. *S. cynosuroides* with no nitrogen additions. II. *S. cynosuroides* with nitrogen additions. III. *P. australis* with no nitrogen additions. IV. *P. australis* with nitrogen additions. Plotted values are least square means. Error bars shown are standard error.




E-6 Comparison of the belowground rooting depth patterns collected in December 2010 in different treatments of salinity and fertilization. I. *S. cynosuroides* with no nitrogen additions. II. *S. cynosuroides* with nitrogen additions. III. *P. australis* with no nitrogen additions. IV. *P. australis* with nitrogen additions. Plotted values are least square means. Error bars shown are standard error.




E-7 Comparison of salinity regime effects on belowground biomass of monocultures at harvesting in December 2010. All samples were collected in 10 cm intervals and homogenized. Plotted values are least square means, and different lowercase letters indicate significantly different values. Error bars shown are standard error ($\alpha = 0.10$).




E-8 Comparison of fertilization treatment effect on belowground biomass of monocultures at harvesting in December 2010. All samples were collected in 10 cm intervals and homogenized. Plotted values are least square means, and different lowercase letters indicate significantly different values. Error bars shown are standard error ($\alpha = 0.10$).

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